Carlos Rafael Costa Gonçalves

Licenciado em Química Aplicada

Synthetic studies on a novel celastrol- fluorescent probe for *in vivo* imaging and novel amination methodologies

Dissertação para obtenção do Grau de Mestre em Química Bioorgânica

Orientador: Prof. Dr. Maria Manuel B. Marques, Investigadora Auxiliar, FCT-UNL

Co-orientadores: Prof. Dr. Nuno Maulide, Professor Catedrático, University of Wien

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Dedico esta coisa àquela coisa que têm demasiados nomes para serem citados.

O truque é desistir... de desistir

The trick is to give up… from giving up

Acknoledgements

Obrigado à Ana, a pessoa a quem devo onde estou e onde vou estar. Obrigado por me guiares e permitires que faça aquilo que eu amo. Obrigado por tudo o que és, o resto, vou dizer-te diariamente pois as verdades são para serem ditas.

Agradeço a toda a FAM que me apoiou durante todo este tempo e um especial obrigado aos grupos e Professores Maria Manuel Marques e Professor Jorge Parola por toda a diversão e apoio prestado.

Obrigado ao Departamento de RMN da universidade FCT-UNL, em especial à Ana Teresa pela simpatia e encanto.

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A special thank to sub- chefe Alex pintoz, to El Berto and, or Professor (tio) Nuno Maulide for giving me the opportunity and believing me.

Resumo

Esta dissertação foi divida em duas partes:

Parte 1 consiste em:

O desenvolvimento de uma nova medologia de aminometilação de alcenos e alcinos . O método consiste na utilização de TFA para promover a formação de um ião iminio, formado apartir de tetramethyldiaminometano, que é posteriormente atacado pelo alceno/alcino. Uma grande diversidade de exemplos com diferentes grupos funcionais e alcenos de diferentes reactividades foi estudado. Em geral o método é bastante resiliente e funcionou com diversos substratos. Um total de 25 aminas secondárias foram sintetizadas , sendo isolados depois de uma protecão com o grupo Boc usando como catalizador ácido sulfâmico. Após dois passos os produtos foram isolados com rendimentos até aos 82%.

Parte 2 consiste em:

O desenvolvimento de uma probe fluorescente, usando um ião flavílio para detecção *in vivo.* De modo a desenvolver um sistema fluorescente para detecção *in vivo* de células cancerígenas tentou-se acoplar o ião flavílio fluorescente com o composto farmaceuticamente relevante – Celastrol. Um novo ião flavílio foi formado com a condensação de 3-dietilaminofenol e uma acetofenona funcionalizada na posição 4' com uma cadeia alquilica com um halogénio terminal. Diferentes caminhos sintéticos foram estudados para conseguir o flavílio substituido na posição C4 do anel pirílio. Foram ainda feitas algumas tentativas de acoplamento entre o flavílio e Celastrol.

Palavras-chave: Aminometilação, Síntese de aminas, Alcenos desactivados, Compostos derivados de flavílio, síntese de compostos fluorescentes, celastrol.

Abstract

This dissertation was divided into two parts:

Part 1

A novel methodology of aminomethylation of alkenes and alkynes was studied. The methodology developed involved the use of TFA to promote iminium formation from tetramethyldiaminomethane, which in turn reacts with a nucleophilic double bond to deliver an amine. A very well-rounded scope was accomplished with examples that range from aliphatic, cyclic and aromatic alkenes. Functional group tolerance was also pursuit with the method showing a great tolerance towards commonly used groups such as halides and esters. A total number of 25 boc-protected amines were synthesized using alkenes with yields up 82 %.

Part 2

The second part consisted on the development of a fluorescence probe using a flavylium ion for *in vivo* imaging. In order to develop a fluorescent system that could be used in *in vivo* imaging for detection cancer cells, the prepared flavilyum was coupled with the theraupeutical relevant Celastrol. A new flavilyum ion was synthesized via condensation of a 7-diethylaminophenol with acetophenone possessing an halogenated alkylic linker at the 4' position. Different pathways were investigated in order to reach and optimize the synthesis of a C4 functionalized flavylium salt that was prepared in three steps. Attempts were performed to couple the prepared flavylium to Celastrol.

Keywords: Aminomethylation, Amine synthesis, Unactivated Alkenes, Flavylium coumpounds, synthesis of fluorescent compounds, celastrol

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Nomenclature and Abbreviations

δ	Chemical shift
φ	Quantum yield
^{13}CNMR	Carbon nuclear magnetic resonance
^{1}HNMR	Proton nuclear magnetic resonance
3D	Three Dimensional
A	Quinoidal base
Ac	Acetyl
ACN	Acetonitrile
AcOH	Acetic acid
Ag_2O	Silver(I)Oxide
AH+	Flavylium cation
ax	Axial
B	Hemiacetal
B16F10	Cancerigenous cells culture
BetA	Betulinic acid
Boc	*Tert*-butyloxycarbonyl
Boc_2O	Boc Anhydride
Br_2	Bromine
Bs	Broad singlet
Bt	Benzotriazole
BX	Alkyl base
C	*Trans*-chalcone
Cc	*Cis*-chalcone
$CDCl_3$	Chloroform-d
CMAP	Connectivity Map
d	Duplet
DCC	N,N'-Dicyclohexylcarbodiimide

DCE	1,2-dichloroethane
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DME	Dimetoxyethane
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
Enal	Aldehyde conjugated with an alkene
Eq	equatorial
EWG	Electrowithdrawing group
Equiv.	Molar equivalents
ER	Electrophilic reagent
EtOAc	Ethyl Acetate
FITC	Fluorescein isothiocyanide
H_2SO_4	Sulfuric acid 98 %
H_3PO_4	Phosphoric acid
HBf4	Fluoroboric acid 48 %
HCl	Hydrochloric acid
Is	Isatin
IR	Infra-red
J	Coupling Constant
KI	Potassium
K_2CO_3	Potassium carbonate anhydrous
LiHMDA	Lithium bis(trimethylsilyl)amide
LiOH	Lithium hydroxide
m	Multiplet
m/z	Mass/charge ratio
MeI	Methyl iodine
$MeNO_2$	Nitromethane
MeOH	Methanol
$MgSO_4$	Magnesium sulphate

MS	Mass spectroscopy
NaH	Sodium hydride
$NaHCO_3$	Sodium Bicarbonate
NaI	Sodium iodide
n-BuLi	*normal*-Butyllithium
NEt_3	Triethylamine
WU	Work Up
NIH 3T3	“normal cell” culture
NMR	Nuclear magnetic resonance
o	*Ortho*
OTBDPS	*tert*butyldiphenylsylil ether
PMB	*para* metoxy benzyl ether
ppm	Parts per million
Rf	Retention factor
ROCS	Shape Similarity for Virtual Screening & Lead Hopping
Rt	Room temperature
s	Singlet
t	Triplet
Tf_2NH	bis(triflyl)imide
TFA	Trifluoruacetic acid
TfOH	Trifluorumethanesulfonic
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMDAM	Tetramethyldiaminomethane
TON	Turn-over-number
Ts	Tosyl group- paratoluenosulfonyl
TsCl	Tosyl Chloride

Preface

It is necessary to explain some of the thoughts that were put into the making of this work.

A Master's Dissertation should be a detailed description of the efforts of a student with a specific task at hand. One expects a concise work with a defined topic about a certain object of study.

It should be coherent throughout, meaning that it should have a beginning, a development section and an end, as if a story book with scientific intent.

A motivation that should be apparent when this type of work is undertaken is that 99.9% of it involves other people, whose help is crucial for its success. This is of the upmost importance because a student should be bound to deliver a work that mirrors all the support given to him/her. This is the biggest reason why I decided to structure the work the way I did.

In the last 11 months, I had the opportunity to spend my time in-between two amazing laboratories with different people and learning processes, which I am deeply thankful for. I knew that I had to decide whether to include both topics with the risk of telling an unconnected story or to choose one in detriment of the other. I did not choose the latter option because I felt that I would easily regret it, and that I simply could not do.

The reason for this text is to give the reader my point of view, the background, on why I decided to elaborate this work in two, equally interesting, parts.

Keeping this in mind, I did my best to make them as similar as possible in terms of work methodology and text elaboration – maintaining the logical connections between concepts throughout the work.

I admit this decision could prove to be problematic. However, it was a well-thought out risk I was willing to take in order not to neglect the amount of research done during this period and sacrifice the quality of the dissertation.

Part one- Aminomethylation of Alkenes with imminium Salts

- From olefins to amines

Scheme Index

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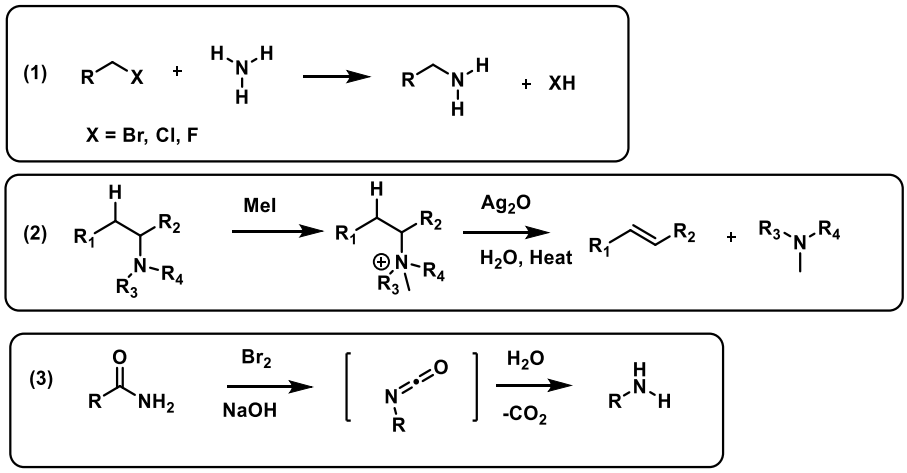
I-Introduction

Amines are one of the most quintessential building blocks that exist ubiquitously all across nature. One can understand their importance by analyzing diverse and up-do-date statistics which underline this very detail. If we take into account the top 200 pharmaceutical products that were sold in retail in the year of 2016 we may conclude that 90% of the drugs have some kind of amine or amine derivative in their structure[1].

In a more specific manner, if we take aliphatic amines into consideration, we find out that 41.9% of the compounds which have reported biological effects, have present in their structure an alkylamino moiety or a derivative thereof.[2]. Aliphatic amines have many and diverse applications that range from dyes[3] to ligands[4] and food flavoring agents[5].



Scheme 1 – (1) – Alkyl halide substitution by ammonia, (2) – Hofmann Degradation, (3) – Hofmann Rearrangement.

The history of the synthesis of amines and its derivatives cannot be thoroughly explained without the introduction of a very particular and important scientist by the name of A. W. Hofmann who, curiously, was the first to use the term “Synthesis” when talking about the formation of styrene and its degradation[6]. His contributions to organic chemistry are countless and immensurable: from all of them, we can highlight the work related to the synthesis of amines which revolutionized the 19th-century chemistry (1858)[7,8],[9]. In between many reports by Hofmann, we can find: the amonium salts synthesis by polysubstitution of alkyl halides with ammonia; the so called Hofmann elimination in whicha quaternary amonium salt is used to yield an olefin and the famous amide rearrangement to amines, also named after him.

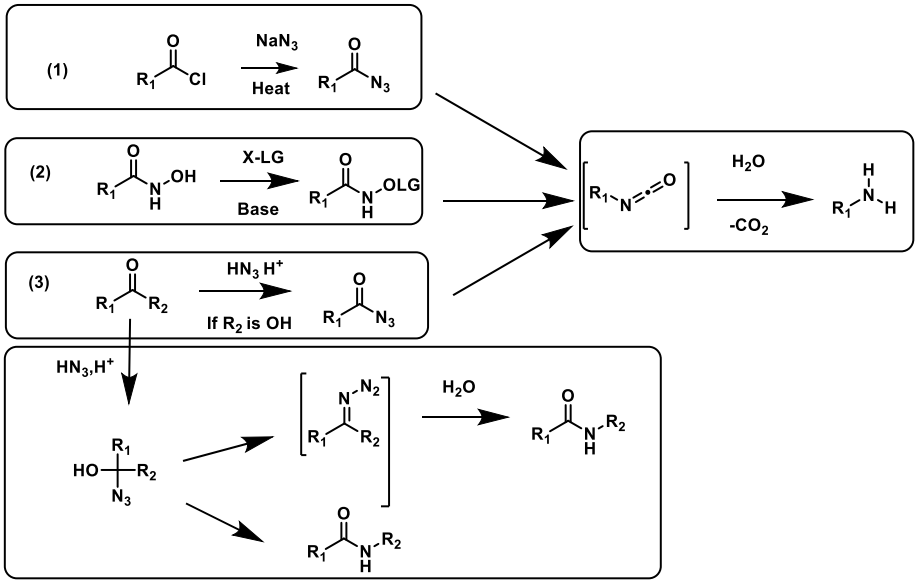
There are major drawbacks associated with the previous reactions, resulting in their scarce use over the years. The main problem of synthesizing amines via alkylation of ammonia with alkylhalides is indeed the overalkylation, which is due to the fact that the resulting product (primary, secondary and then tertiary amine) is more nucleophilic than the starting amine. Istead of being used in the synthesis of tertiary amines, the previous reaction is useful in the formation of quaternary amine salts which are used in many areas such as phase transfer catalysts or as surfactants. [10]

As shown before, these salts can also give the correspondent tertiary amine by elimination using silver oxide(I): this reaction is not usually employed in the synthesis of amines *per se* but, instead, it is used to prepare less substituted alkenes; which are generally more difficult to generate than their more substituted counterpart, due to their lower thermodynamic stability (follows Hofmann rule).

As expected, there have been tremendous developments of the Hofmann rearrangement since its discovery almost 140 years ago [11],[12].Bromine itself has, since then, been replaced by other sources of halogens such as NBS or TCCA or hypervalent iodine reagents are employed, if base-sensitive substrates are used [13].

The Hofmann rearrangement is intrinsically related to two other known rearrangements: the Curtius and Lossen, both mechanistically and strategically(scheme 2). They all generate a reactive N-X amidate which then evolves into an isocyanate intermediate. The oldest one of this group is the Curtius rearrangement where an acyl azide is formed by nucleophilic acyl substitution from an acyl chloride by an azide, this in turn undergoes loss of X^-, which in this case is N_2, to unveil the isocyanate moiety [14].The Lossen rearrangement instead, involves the use of hydroxamic acids as starting materials; this means that there are no azide-containing intermediates, species which are difficult to manage. Despite this fact, the harsh conditions necessary to generate the leaving group along with other drawbacks make up the reasons why this rearrangement is not often used in practical synthesis [15].

Another similar reaction to the previous ones is the Schmidt rearrangement, whose main difference consists in the reactions starting materials: usually it is performed on ketones and not on carboxylic acid derivatives [16]. There have been two proposed mechanistic pathways to explain this reaction: the Bayer Villiger mechanism and the more generally accepted Beckmann mechanism[17]. Allthough the reaction is used normally to generate amides from ketones, amine formation is possible if a carboxylic acid is used as starting material.

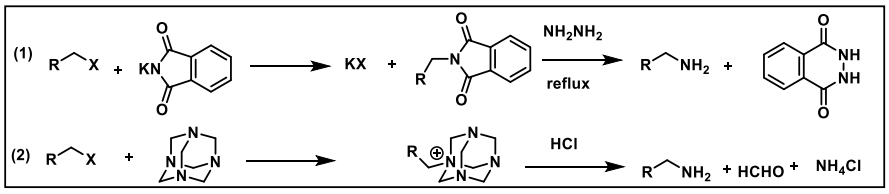


Scheme 2 – (1) – Curtius Rearrangement, (2) – Lossen Rearrangement, (3) – Schmidt Rearrangement.

A noteworthy similarity between the previous four rearrangements is that they all access the primary amine moiety, which is remarkable due to inherent difficulties in their synthesis from alkyl halides, as mentioned before.

Some years after those discoveries, a student of Hofmann, Sigmund Gabriel, proposed a variation that involved the use of potassium phtalimide as a ammonia surrogate[18], to unveil the primary amine moiety after hydrazinolysis. One of the problems in this method is exactly this last step, where the "deprotection" of the phtalamide may require harsh conditions, even though there are some alternatives to overcome this difficulty[19].

A better path to get to the primary amine, which rectifies some of the issues encountered in the Gabriel synthesis, is the so-called Delépine Reaction[20]. Instead of using the phtalamide salt, hexamethylenetetramine is used to get to a quaternary amonium salt, which is subsequently hydrolyzed in acidic medium. In comparison, this reaction is far more selective, leading to less side products and milder conditions.



Scheme 3 – (1) – Gabriel Synthesis, (2) - Delépine Reaction.

An important detail that should be taken into consideration is the applicability of these reactions from an industrial point of view, due to the fact that the relative importance of a reaction should be measured also by the feasibility of its execution in large scale. So far, the meth-

ods that were introduced are rather missing in this regard; because of the harsh conditions necessary, which are difficult to perform in large scale, because of difficulties in managing some reagent (i.e. hydrochloridric acid is corrosive)[21].

If we take a look at the most favorites methods from the chemical industry perspective, reductive amination is by far the most prefered and used procedure. This important tool consists in the condensation of an amine with an aldehyde or ketone to generate an iminium ion, which in turn is then reduced to yield the amine. Even though there are examples where the reaction is performed in a stepwise manner(i.e. where the imine/oxime is isolated before the reduction), the vast majority of reductive aminations are performed in a one-pot manner due to trouble purifying the intermediates.[22].

The range of substrates that can be used are remarkable and speaks volumes about the applicability of the method. Even when unactivated carbonyl compounds or sterically hindered amines are used, the reaction can still be employed, sometimes with additives such as molecular sieves (to shift the equilibrium toward the iminium ion formation) or Lewis acids (to increase the electrophilicity of the carbonyl compound). Though being a really popular reaction, there are nevertheless, some problems that need to be addressed, as for example: the possibility of side reactions (Mannich-type condensations and others) or reduction of the carbonyl species instead. All in all, reductive amination is still one of the most direct and simple ways to prepare amine moieties[23].

A factor that has been completely overlooked so far is the stereochemistry that can be embedded in the structure during the synthesis of the amine itself. This possibility attracted great attention because of the huge impact of the synthesis of chiral amines[24], which have important pharmaceutical value. From the examples cited above, the one most employed to generate chiral centers is the reductive amination, which is able to deliver high enantiocontrol in its reduction step[25], in most cases. This leads to the use of this reaction almost routinely to access a chiral amine functionality.

Another relevant reaction which is useful in delivering in a stereoselective fashion amine moieties is the aza-Morita-Baylis-Hilman reaction, that, as the name suggests, is a nitrogen variation of the Morita-Baylis-Hillman reaction[26] . The line of thought, in this case and similar reactivities, is that by including a C-C bond formation when talking about amine introduction into a molecule is that we can synthesize chiral amine from arguably one of the most simple substrates: the alkene .

The complexity of this reaction, that utilizes the electrodeficiency of an alkene that has its polarity inverted thanks to the action of a tertiary amine or phosphine, and the use of a al-

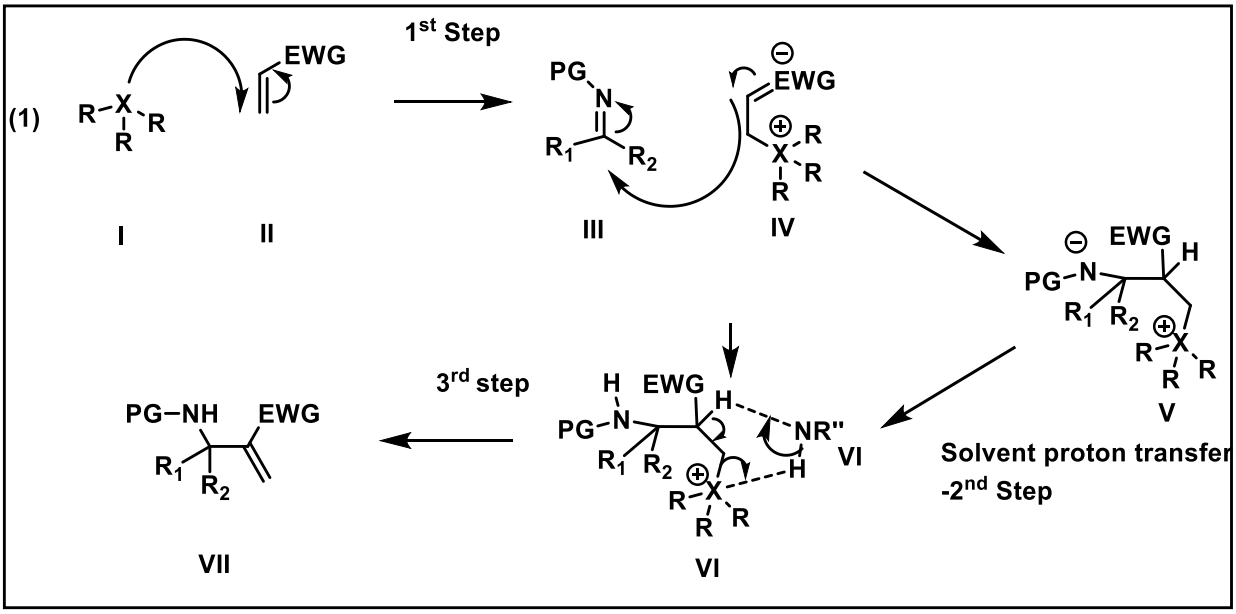
dimine, is interesting due to the high degree of functionalization that can be obtained. The potential that is associated with the Baylis-Hillman is high and, for that reason, a great amount of resources are being used to develop it. The major drawbacks involve: long reaction times (in mild conditions) and stereochemistry somewhat difficult to control, both of which are being slowly overcome over the years.

From a mechanistic point of view, which is quite impressive and intricate by itself, led to much discussion throughout the years because of the possibility of enhancing the yield and lowering the reaction time, after knowing, in a concrete manner the reaction's pathway. There are actually two proposed mechanisms: one by McQuade[27] and the other, which we have chosen to elaborate, by Aggarwal[28].

Concerning the mechanism proposed by Aggarwal (scheme 4), the first step consists in the conjugate addition of a Lewis base (phosphine or tertiary amine) (I) to an electrodeficient alkene (II) to generate a zwiterionic species (IV). Intermediate (VI) is formed by the Mannich-type addition to the protected aldimine (III) (normally a tosyl protected aldimine). The last step (the elimination/recovery of the promoter) is thought to be the rate determining step at low concentrations of the product.

Aggarwal's proposal consists in the autocatalysis by the product (VII) itself, which acts as a hydrogen bond donor. Since this type of reactions normally occur in acidic medium and with a protic solvent that meant that solvent interactions could not be discarded (MeOH hydrogen bonding is possible). The autocatalysis leads to a change of the RDS from 3rd to 2nd step when the product concentration is high enough for the transference of the proton to be probabilistically more facilitated. The solvent proton exchange that leads to the formation of (VI) is then considered the RTS.

The Kinetic isotopic Effect (KIE)[29] calculations are congruent to the mechanism proposed before: primary KIE for the 3rd step at low conversion. It is also important to note that the direct elimination from the amidate (V) to product (VII) was ruled out by geometric constrains.



Scheme 4 – (1) Aza-Morita-Baylis Hilman mechanism proposed by Aggarwall.

The potential that this reaction exhibits is truly remarkable and can be attributed to its scalability. Other positive aspects are, for example, the atom economic nature and commercial availability of the starting materials (even more evident in the original Morita-Baylis-Hillman reaction, where aldehydes are used). It is unfortunate, though, that only electrodeficient alkenes surrogates can be used in this chemistry, which greatly limits the scope.

Alkenes are amongst the most attractive starting materials for chemical synthesis, mostly because of their characteristics and relative abundance. The ease from which they can be accessed in both large and laboratory scale makes them one of the most important scaffolds. If we take a look around, we can see numerous examples of their usefulness that range from polymers, that aere used to make almost everything surrounding us to other utility fabrics.

Nowadays most of stock olefins are generated by steam cracking of hydrocarbons and this fact will held true in the upcoming years due to lack of investment in new technologies. The decrease in crude oil reserves and the acknowledgment of global warming is not alarming the alkene industry, partially because of their high economic viability[30] . This fact resonates with one the characteristics that make them so interesting – a general mild chemical inertness. They are generally non-polar, hence, inert to most powerful bases, milder oxidants and, most importantly, other polar functional groups allowing them to be manipulated in their presence [31] .

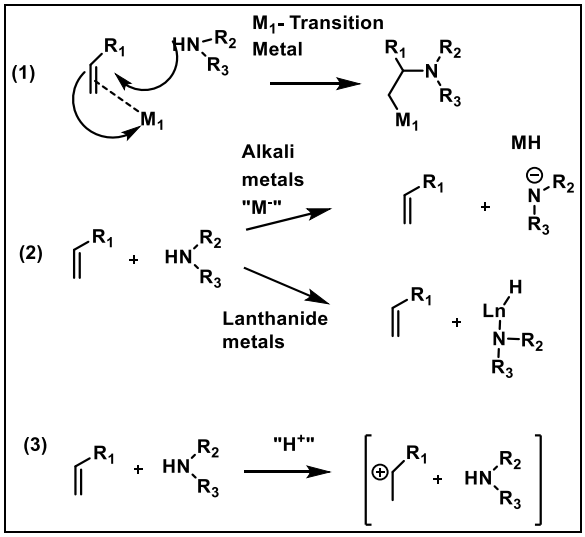
High chemoselectivity can be achieved when specialized reaction conditions are employed and, for that reason, from a strategic chemical synthesis point of view, they are sought

after especially in late stage functionalization, when the presence of other functional groups is problematic.

Alkenes are involved in the synthesis of amine, and the chemistry involved is quite important: hydroamination, which, as most reactions of addition cross alkenes, comes from the fact that a hydrogen(hydro) and amino(amination) group are both added to the alkene. One curious note is that the only exception to this nomenclature (Hydroboration, hydrobromation, etc..) is the so called thio-ene reaction, that has a retained name because it was discovered before the others. Back to the topic, there are two different regiochemical outcomes depending on the site where the addition occurs: Markovnikov-type products are obtained if the hydrogen ends up in the less substituted position, and anti-Markovnikov-type if the regiochemistry is the opposite one[32,33].

Although the concept of hydroamination is tremendously useful, there are a few issues that prevent it from being the favored method to synthesize amines yet. The electrostatic repulsion between the π electrons of the olefins and the lone pair of the nitrogen atom is immense being both electronrich and, for this reason, the reaction does not occur. Concurring with this fact, a [2+2] addition is forbidden by orbital symmetry rules, meaning that increasing the reaction temperature would not increase its occurrence(only known 2+2 expection is using allenes). It has been also reported that there is a huge difference between the py orbital of the C=C bond and the sigma N-H that renders the cycloadditon inoperable [34].In terms of thermodynamic values, the reaction itself is only slightly negative in enthalpy as well as in entropy meaning, that, once again, the temperature does not favour the transformation[35].

Several strategies have been investigated to develop effective hydroamination reaction. We can group them in 3 different groups: use of transition metals as catalyst [36]for activation of the N-H bond by alkali metals or lanthanides; Browsted acid catalyzed hydroamination.



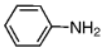
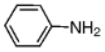
Scheme 5 – (1)- Mechanism of hydroamination using transition metals, (2) – hydroamination using alkali or lanthanide metals, (3) hydroamination using Bronsted acids as catalyst "H^{+}".

In case of transition metals as catalysts in hydroamination, there are several mechanisms of action that have been proposed in literature, depending on, for example, the transition metal or whether a cocatalyst is used (i.e. Bronsted acids). The most common situation is a nucleophilic attack of an amine on the metal–alkene complex. This step determinates the regioselectivity which is normally a Markovnikov regioselectivity due to the increased stability of the ensuing carbon metal bond. The activation of the alkene by the transition metal is the key step, otherwise no reaction would occur [37].

When talking about base-catalyzed hydroamination, we can divide it in two subclasses depending on the type of catalyst: the cheap and abundant alkali metals and the rare elements such as lanthanides or rare earth metals. In order to overcome the high energy difference between the orbitals of the substrate, an amide is formed from the amine in order for it to be reactive towards the alkene. This is normally achieved with high temperature and pressures in the presence of strong bases such as akyllithium, for example. When using the lanthanide catalyzed reactions the system is more susceptible in case of intermolecular hydroamination, where a concerted 4 membered transition state between the N – Ln – H and the alkene has been proposed [38].

A simple table that is helpful in understanding why these types of reactions are not easily feasible on a large scale is the following:

Table 1 – Base Catalyzed hydroamination of olefins with alyphatic amines and ammonia[39].

Olefin	Amine	Catalyst	Temperature [°C]	Pressure [bar]	Amine Yield [%]			TON	TOF [h^{-1}]
					primary	secondary	tertiary		
═	NH_3	Na	175–200	800–1000	26	26	14	9	1
	NH_3	$CsNH_2/RbNH_2$	101	90–110	28–34	2–3	1–2	3	1–3
		Na, Pyridine	100	28–38	–	–	77–83	17	5
		Na, Pyridine	100	41–55	–	–	80	25	8.3
	n-C_4H_9-NH_2	Na	200	800–1000	–	–	75	2	–
	n-C_4H_9-NH_2	$LiNEt_2$/TMEDA	130–150	150–250	–	54	45	56	1.5
		Na/NaH	225	1000	–	–	28	<1	–
		$LiNEt_2$/TMEDA	140	70	–	–	83	14	1
		$NaNH_2$	275	41–55	–	75	2	10	2
	NH_3	Na	250	800–950	82	8	–	12	0.65
	n-C_4H_9-NH_2	Na	250	860–1000	–	36	–	1	0.06
		$LiNEt_2$/TMEDA	150–170	70–90	–	–	10[a]	8	0.3
		$NaNH_2$	330	50	–	6	–	0.8	2

We can clearly see that despite the very harsh reaction conditions, the yields are very low. It is also important to note that for a catalytic reaction to be considered feasible in an industrial scale, its catalyst must have a TON (Turn-Over Number) higher than 1000. In reality what happens, to the best of our knowledge, is that no catalyst for intermolecular hydroamination has a TON higher than 500 [39].

The last type of catalysis involves the use of Bronsted acids (scheme 5) and it is the one that has been studied the least. The inherent problem associated with it is that the acid catalysis can react both on the olefin and the amine moiety, since both are nucleophilic partner, leading in case of protonation of the amine moiety to the deactivatation of the amine in form of the ammonium salt. The amine is actually more basic than the π system of the olefin, in almost any reaction condition, the salt of the amine is formed making the reaction not fruitful[40].

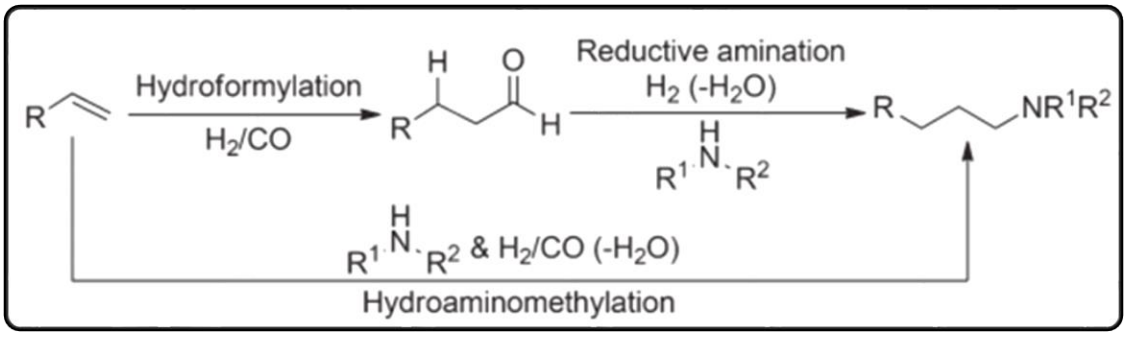
In general, the addition of amines to alkenes results in a Markovnikov regioselectivity, which is the least interesting product from an industrial point of view [41]. This is exemplified by the fact that in 1993 the anti-Markovnikov product was considered one of the top 10 challenges in catalysis [42]. One obvious issue common to all the hydroamination approaches that were presented is that all the reactions involve very sensitive reagents and conditions that can greatly affect, for instance, the reproducibility.

There are, nevertheless, other problems which are common to all these methods involving catalysts. One of them is that the product itself, the amine, can act as a ligand and poison the catalyst: one solution is the use of superstechiometric amounts of catalyst, defeating the purposes of the concept of catalyst itself. It is quite ironic that, the ability of the amine to act as ligand for very complex catalysts systems, also makes it harder to synthesize them. Furthermore, regarding the hydroamination, although the transformation is formally 100% atom efficient, the reactions reported in literature have a large excess of olefin in comparison to the amine [34]. In addition, most catalytic systems are deeply substrate specific, and usually do not allow for extrapolation for a wide range of applicability.

The synthetic community has therefore realized that there are several problems related to hydroamination; however, olefins as feedstock for synthetic reactions are still very appealing. One way to circumvent the problems is to simply transform the olefin into another functional group in a first moment and, in a second step, functionalize it to the amine. Following this line of thought, one can exploit reductive amination, which is, as we have seen before, the preferred method for the synthesis of amines. Starting from an olefin one could consider the transformation of the alkene into an aldehyde (the so called hydroformylation[43]) and use this moiety to introduce the amine functionality.

The production of aldehydes from alkenes is a process that has been explored thoroughly since its invention. The reaction requires high temperatures and pressure besides the use of transition metals. Usually the yields are quite good and this reactivity is successfully adapted to industrial processes. For that reason, in 1995 alone, production reached 6.66 10^6 [44].

The process including the reductive amination after hydrocarbonylation, is formally referred to as hydroaminomethylation: a process that transforms an alkene into the homologated amine. This reaction was discovered in the 1950s by Reppe[45] and it is a very powerful way to generate amines. It has quite an intricate catalytic system that must fulfill several requirements. Originally, the hydroformylation and reductive amination were executed separately, however, over the years, one pot procedures have been developed.



Scheme 6 – Scheme of Hydroaminomethylation[46].

There has been quite a lot of research done in this field with different catalytic methods being optimized. One of the most utilized involves the use of Rh-based catalysts to perform the formylation and, at the same time to catalyze the reduction with the simple molecular hydrogen[47].One interesting note is that the regioselectivy can be mildly controlled by the type of ligand that is[48]. Regarding the drawbacks of the reaction, they consist in the difficulty in controlling the reduction of the imine and compared to reduction of the initial aldehyde. Overalkylation can also happen especially when primary amines and ammonia are employed [46].

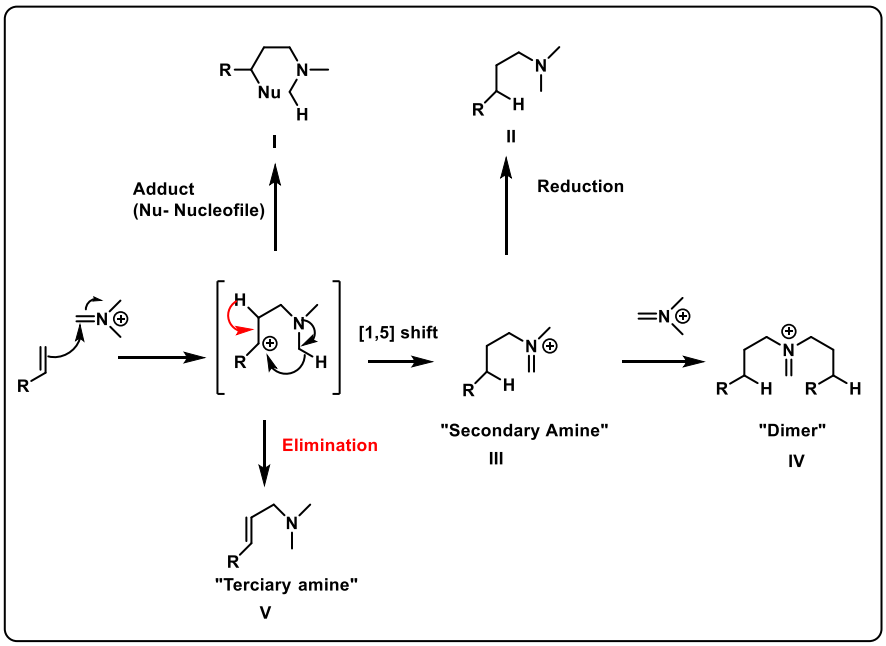
If we take some of the abovementioned concepts and connect them, an old reaction comes to mind: instead of having an alkene, transforming it into an aldehyde and then insert the amine, we can think about the direct reaction between alkenes and aldehydes. This transformation is called Prins reaction and was first reported in 1913[49]. It consists of an acid catalyzed nucleophilic addiction of an olefin to an aldehyde. Usually, a strong protic or Lewis acid is required for the reactions to work.

The harsh conditions combined with the presence of water in the reaction can lead to different products. One of the most common consists in the addition of an exogenous nucleophile, usually water, to the formed carbocation (I). This very same carbocation can suffer elimination to give the allylic alcohol (V). Because of the nature of the reaction, other reactions such as rearrangements can also occur, meaning that the reaction conditions must be carefully controlled to avoid several byproducts[50] .

A few years later a new variant of the Prins reactions was reported and its relative importance for the general theme of this work is immense. More precisely in 1954 Dickert reported a type of Prins reaction working on imines rather than aldehydes: the aza-Prins. The actual reactive species has to be a cationic iminium in order for the nucleophilic attack of the alkene to happen. He also found a different product derived from what appeared to be a subsequent [1,5] hydrogen shift occurring from an α-methyl group of the tertiary amine, resembling the mechanism of the Sommelet reaction [51],the iminium ion is then hydrolyzed to afford a secondary

amine(III). As a matter of fact we can also see this type of transformation as an *ene* reaction [52]. The main difference is whether the mechanism follows a cationic stepwise process or is concerted. Furthermore, this second iminium ion can react once again to yield a sort of "dimer" which can also be hydrolyzed (IV).

The first examples of this transformation involved the use of acetic acid as solvent and sulfuric acid as additive to generate the iminium specie *in situ* from dimethylamine and formaldehyde (scheme 7). This was in fact the favored method for the reaction to proceed. Other solvents where employed but with scarce results: i.e. formic acid resulted in another side product – the reduced tertiary amine(II) due to the very high temperatures that are usually employed in this sort of reactions together with the fact it is a reducing agent[53].

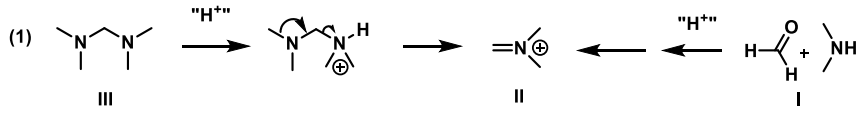


Scheme 7 – Proposed mechanism of the aminomethylation with most of the possible products.

These type of reactions were taken up again years later by Cohen[54], resulting in corrections of some results and better characterization of some compounds that he found to be wrong in the original report[55].

The misassigned structures in question were secondary amines that were confused by tertiary amines by Schmidle .This turned out to be a major problem, because most articles related to this subject in the 50s have problems inherent to the characterization part mostly due to lack of tools to properly assist them. Cohen used a different way to perform the reaction: he used the known Eschenmoser's salt (II), a stable and isolable iminium specie (whose name comes from the famous chemist Albert Eschenmoser, who synthesized and optimized the procedure in order synthesize this salt, amongst other impressive feats). He also tried to use another

strong acid, i.e. phosphoric acid, as a replacement for sulfuric acid, nut this did not show any significantly improved outcome of the reaction. The most important alternative way to generate the iminium salt was the use of tetramethyldiaminomethane which, in acid medium, decomposes to the necessary cation specie required for the reaction. The significance of this last example is exacerbated throughout the work due to its utilization and further modifications that are done to the diamine molecule.



Scheme 8 – (1) Different method to generate the iminium specie. (III)Tetramethyldiaminomethane, (II) Eschenmoser's salt equivalent (iodine or chlorine as a counterion), (I) dimethylamine and formaldehyde in a presence of a strong acid.

As for the olefins that are normally used in the reaction, most examples use "activated olefin", which means cyclic olefin whose geometric constraint or cation stabilizing effect leads to increased reactivity (norbornene, styrene for example). Notwithstanding, the yield ranges between 10% and 50%, which is considered low. There are also a few examples of terminal aliphatic alkenes whose products were isolated in an even lower yield. A major limitation about this reactivity is the total absence of examples working with internal olefin besides the abovementioned cyclic ones.

Another important factor is the procedure to isolate this specie: it is quite narrow. Distillation was the only one that was successfully reported. This can be attributed to the fact that amines, in general, are difficult to purify, especially aliphatic amines that are very apolar.

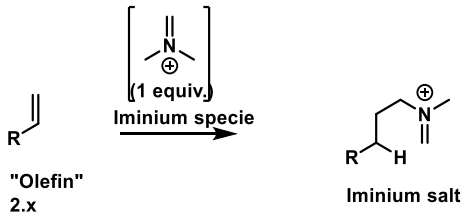
The lack of research on this very interesting topic is remarkable and the scarcity of reports in the chemical literature are testament to it.

II-Discussion

II.1-Methodology for Aminomethylation

II.1.1-Literature reproduction

Because the aminomethylation area of work is considered to be fairly unexplored the first logical move is to careful confirm the some of the assumptions which are related to this type of reaction.

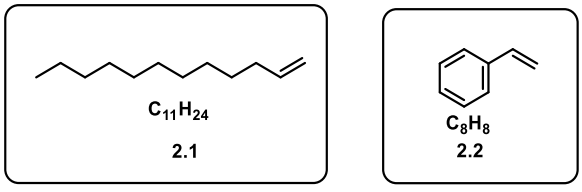


Scheme 9 – Representation of the aminomethylation reaction using a generic olefin (2.x) and a iminium specie that generates a iminium salt after nucleophilic attack of the olefin and a [1, 5] hydrogen shift.

As the name suggest the transformation consists on the addition of a methylene amino group to an alkene. This reaction is reported to occur in highly acidic conditions and is mechanistically very interesting. The proposed mechanism involves the nucleophilic attack of the olefin to an iminium salt. After this step a supposed intramolecular [1, 5] hydrogen shift occurs leading to an iminium ion once again. Arguably the most important initial disclosure of information about this thematic, the aminomethylation, was Cohen's original paper[54].

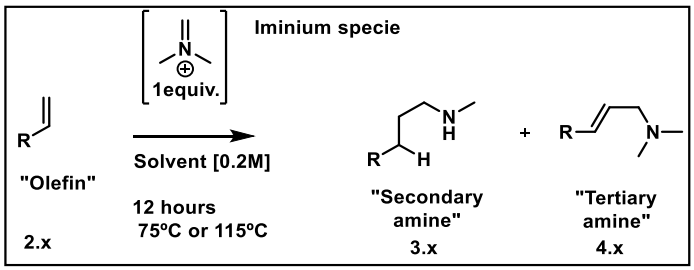
It was then decided to meticulously study, analyze, and finally reproduce the results. The reactions were then repeated exactly within the same conditions, the only difference being the purification procedure, which we changed: the original one involved a distillation. There are a couple of problems with this process: the first one is that it is most suitable with high amounts of crude material and throughout this work we decided to work with 1 mmol; the second one is related to the final goal of this investigation, which is the pursuit of an easy and reproducible methodology, this means that distillation is not an appropriate purification technique.

To begin the study we choose two representative olefin of different type to try and optimize the reaction: undecene and styrene. Both being cheap, easily available and well known in the literature with similar chemistry.



Scheme 10 – 2.1- Molecular structure of Undecene, 2.2- Molecular structure of Styrene.

In the beginning we wanted to compare the two main methods that were reported for the aminomethylation : the use of Eschenmoser's salt in an aprotic solvent like acetonitrile (Conditions A) and the use of tetramethyldiaminomethane in acidic medium (Condition B). We also took into account that the Eschenmoser's salt could be used in a protic, polar solvent, i.e. acetic acid (Condition C). These were the conditions applied when repeating the reactions; however, an easy technique was in need to correctly identify and isolate the products from the mixture that was formed in the reaction.



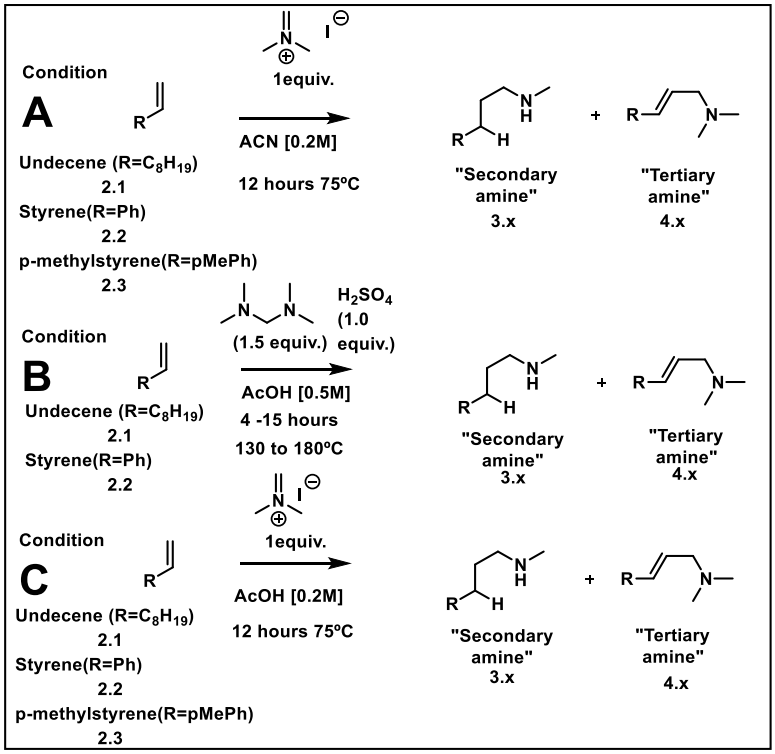
Scheme 11 – General schematic of the aminomethylation reaction.

Briefly the aminomethylation reaction involves the electrophilic substitution of the alkene with an iminium salt that can be generated in situ in acidic conditions. Normally a polar solvent is used in this type of reaction along with high temperatures whose promote the transformation. The previous two factors can lead to several byproducts and for that reason a competent purification procedure was needed to fully study this reaction.

The first approach in order to improve the purification was an acid-base extraction, which is the favored method in purifying amines out of complex mixture, thanks to their possibility of being protonated /deprotonated .Following this line, the solution was quenched with NaOH 50%, as in the original paper, and then extracted. Following this step, the collected organic phases were diluted with HCl and the aqueous phase was collected this time, finally the aqueous phase was basified with NaOH pellets one last time and extracted again, to collect the organic phase. The combined organic phases were dried with K_2CO_3 instead of $MgSO_4$ because of the nature the products (amine), which in presence of residual acid from $MgSO_4$ Can from ammonium salts and remain stuck on the drying agent leading to unnecessary loses .

To our surprise, the crude quantities recovered after this standard work up procedure were very low and so were the ^{1}HNMR spectra. In order to be able to compare results, we also proceed in doing the work up exactly as the original paper suggested, except from the distillation, and the crude quantities were far superior in comparison with the acid base extraction. In order to quantify and discuss the results we resorted to ^{1}HNMR analysis with 1,3,5 trime-

toxybenzene as an internal standard, this lead to results which were comparable to the ones initially presented by Cohen[54].



Scheme 12 – Initial reactions performed by Cohen et all[54], reaction were conducted in 0.1mol scale and the purification procedure involved factionary distillation. Products are represented as 3.x and 4.x if they derive from olefin 2.x.

Table 2 – Entry's 1-5 literature results, entry's 6 – 9 reproduction of the literature results (light blue). [a] ^{1}HNMR yield Condition A –Eschenmoser´s salt (1 equiv.) in acetonitrile [0.2M] at 75 °C per 12 hours/ Condition B – tetramethyldiaminomethane (1.5 equiv) + Sulfuric Acid (1 equiv..) + Acetic Acid [0.66M] at 130°C per 4 to 15 hours / Condition C – Eschenmoser's salt (1 equiv.) in acetic acid [0.2M] at 75°C per 12 hours.

Entry	Olefin	Condition	Amine Yield (%)	3.x (%)	4.x (%)
1	Undecene	B	37	8	91
2	Styrene		63	8	91
3	Styrene	A	67	24	76
4	p-methylstyrene		59	68	32
5	p-methylstyrene	C	44	31	69
6	Undecene	B	40[a]	8	91
7	Styrene		58[a]	8	91
8	Styrene	A	43[a]	7	76
9	Styrene	C	30[a]	66	33

The results from the literature reproduction were quite interesting: the first things that immediately meets the eye is the comparison of the yields on the reproduced reactions, which do not show a significant difference with the ones reported by Cohen[54], the only exception being condition C (entry 5 and 9), the explanation comes from the fact that two different olefins are used: para methyl styrene has a more nucleophilic double bond, compared to styrene, due to the activating effect of the methyl group leading to a higher yield and different product distribution.

As expected, the yield with the aliphatic alkene is lower in comparison to the other electronically activated alkenes.

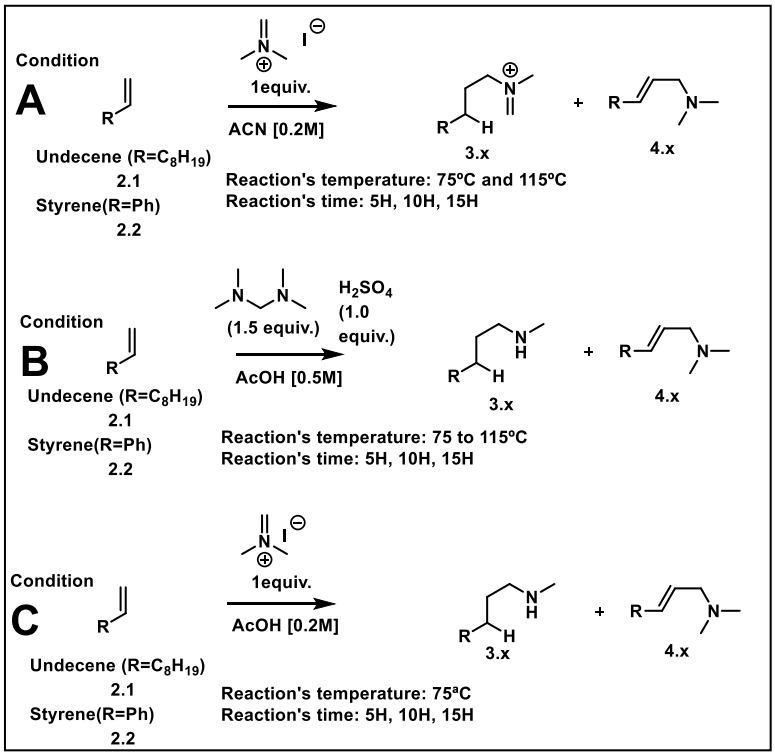
One point of discussion is the quantity of the tertiary amine (4.x) which, as it was discussed before, is the product of elimination. From the literature results, we can infer that a higher amount of elimination product comes from the use of Eschenmoser's as a source for the iminium specie. One other reason would be the polarity of acetonitrinile (condition A) that favors the formation of the elimination product[56] .In regard to condition C, if we take the dielectric constant of AcOH into consideration, it appears that it is less polar than acetonitrile, which is untrue. In this topic, the experimental results from the repeated reaction have contrary information from what was reported. It was found that when acetic acid was employed, a much higher amount of tertiary amine was identified. As far as reasoning is concern, although the acetic acid's dielectric constant is lower than acetonitriles, its reported relative polarity is actually higher, thus justifying the results[57].

A necessary remark is the fact that the comparison of the results is being made on isolated yield (literature results) and ^{1}HNMR yield (reproduced results) and, obviously, one is more trustworthy than the other. We also had some problems when using the ^{1}HNMR to calculate the yield, as it will be discussed ahead.

Although less visible, the effect of temperature in the reaction can also be commented on. From the experimental data, we can relate a higher yield of total amine products to high temperatures, which contradicts the known principle that says that the thermodynamic product, the unsaturated amine, is usually favored by high temperatures. The [1; 5] shift seems to be preferred in these conditions. *Sin*ce both temperatures are already quite high (75°C and 115°C) we needed to investigate if the yield increases incrementally with temperature. This was the first step we took towards the optimization of a possible method. We decided to screen a wide range of temperatures with the intention of finding the mildest condition possible for the three different conditions we selected, before that, and initial study which is of the upmost importance is related to the reaction's duration.

II.1.2-Study of the reaction's time

A very important study that was done is related to how long the reaction takes. It was imperative to know the reaction´s conversion especially if an acid-base work up is employed, because no starting material (olefin – 2.x) will be seen in the final organic phase that is analyzed. We then selected 3 different reactions' times and measured the conversion by comparing it to the reference material in the ^{1}HNMR spectra. This reactions were extracted with a normal work up (quench NaOH and extraction with diethyl ether) to compare.



Scheme 13 – Study of the reaction time of all Conditions(A, B and C). Conversion was calculated using 1,3,5 trimetoxy benzene as a ^{1}HNMR internal standard.

Table 3 - Reaction time studies. All reaction were conducted on a 1 mmol scale of undecene. Condition A – Eschenmoser´s salt (1 equiv.) in ACN[0.2M]/Condition B – tetramethyldiaminomethane (TMDAM) (1.5 equiv.) solvent [0.66M] + sulfuric acid(1. equiv.) / Condition C – Eschenmoser's salt(1 equiv.) in AcOH [0.2M]. Work quenched with NaOH (50%) + extraction with diethyl ether. ^{1}HNMR internal standard (1, 3, 5, trimetoxy benzene).

Entry	Condition	Temperature[ºC]	Time[h]	^{1}HNMR conversion (%)
1	A	75	5	30
2			10	75
3			15	90
4		115	5	40
5			10	80
6			15	100
7	B	75	5	40
8			10	85
9			15	100
10		115	5	45
11			10	90
12			15	100
13	C	75	5	35
14			10	70
15			15	95

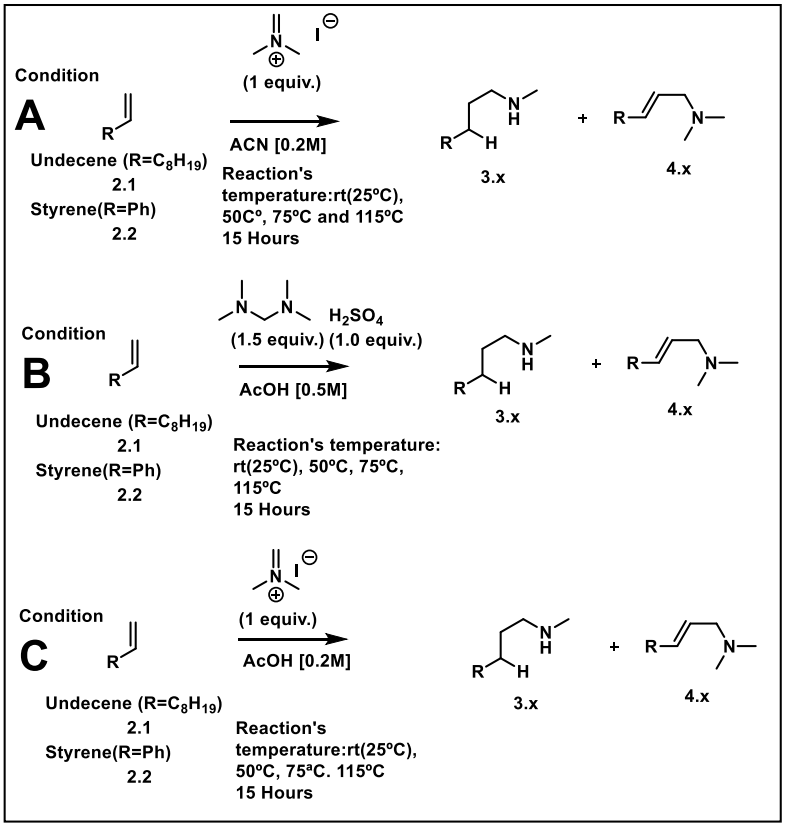
From the data collected we can figure out that there is a direct relationship between the conversion and the temperature at which the reaction occurs. Even though this happens in most cases, we decided to set the reaction time to 16 hours in order to make sure that a full conversion was met in any condition. We used ^{1}HNMR conversion to compare, in particular the typical alkene protons to quantify the values.

In the intermediate times (10 hours) we see the same trend as before, with the last hours of the reaction having much less effect on the conversion of the starting material in comparison to the initial ones.

From this point on we used the reaction time of 16 hours in all experiments.

II.1.3-Optimization of the reaction's temperature

We could not exclude any of the methods that were experienced so far because all of them had some kind of positive aspect towards them, either by the type of solvent such is the case with ACN or by the potential of the conditions like with TMDAM. Having set the optimal reaction time we moved on to the next parameter to study: the temperature. Since conditions A, B or C showed a similar reactivity so far we decided to bring them on in parallel in these studies.



Scheme 14 – Study on the effect of temperature in the reaction. ^{1}HNMR was calculated using 1,3,5 trimetoxibenzene as an internal standard.

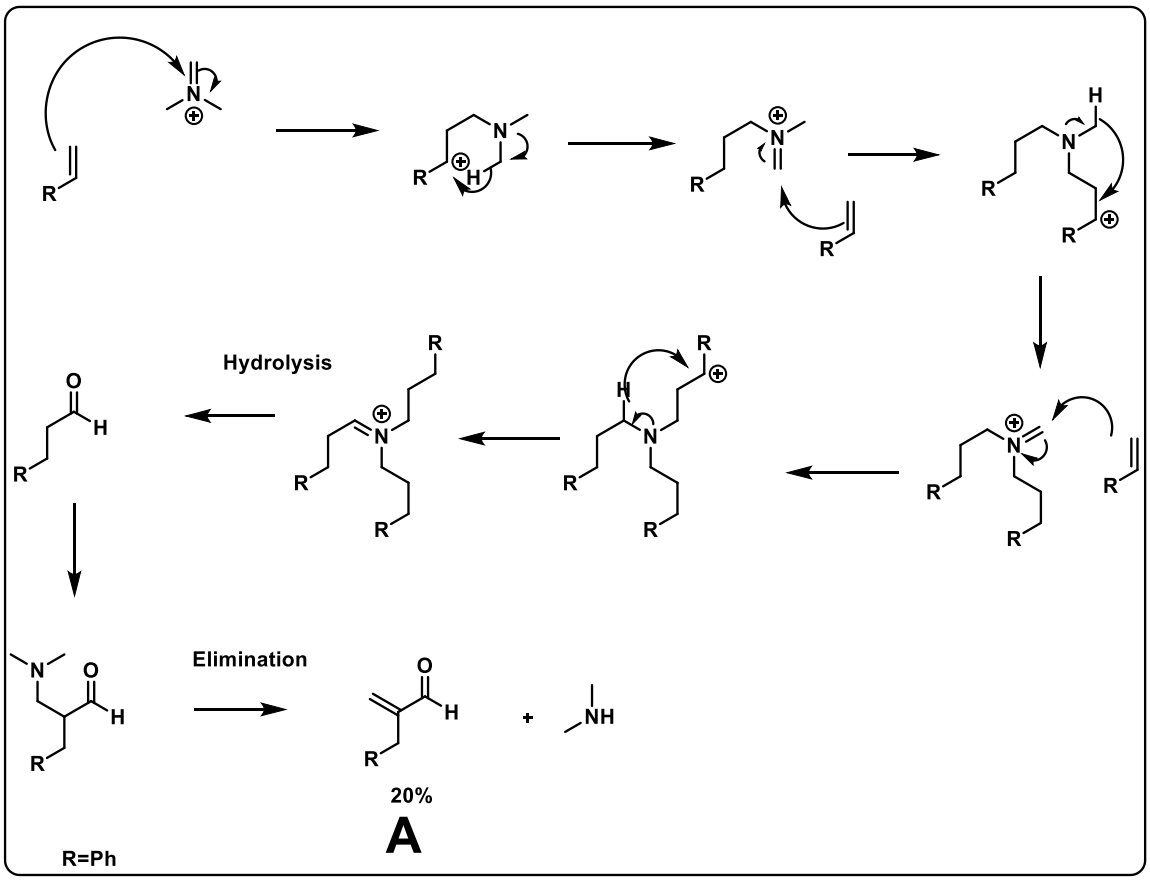
A necessary remark that is needed for the better comprehension of the optimization of the method is that, crude quantity was used as a measure of quantity of different compounds that were created during the reaction and by no means does it accurately describe the quantity of product. The decision to use this parameter in the discussion of this work was to improve the information about the reaction itself leading to a more instructive analysis of the problems and results.

Table 4 – Temperature optimization of 3 possible methods (Condition A, B and C). All reaction were conducted in a 1 mmol scale Condition A –Eschenmoser´s salt(1 equiv.) in ACN[0.2M] at 75ºC/Condition B –TMDAM(1.5

equiv.)in AcOH[0.66M] and H_2SO_4 (1.5 equiv.) for 15 hours / Condition C – Eschenmosers salt(1 equiv.) in AcOH[0.2M] for 15 hours. Acid Base extraction was used. [a]Average was used when more than experimental result was obtain.

Entry	Electrophilic species / Condition	Olefin	Temperature [°C]	Solvent	4.x	3.x	Crude quantity mg (^{1}HNMR yield, %)
1	B[a]	Styrene	115	AcOH	traces	99	170 (dimer)
2	B[a]	Undecene	115	AcOH	traces	99	180 (50)
3	C	Styrene	115	AcOH	9	91	110
4	A	Styrene	115	ACN	9	91	45
5	A[a]	Styrene	75	ACN	20	80	80
6	B	Styrene	75	AcOH	traces	99	110
7	C	Styrene	75	AcOH	traces	99	60
8	A	Undecene	75	ACN	8	92	50
9	C	Undecene	75	AcOH	33	66	45
10	B	Styrene	50	AcOH	traces	99	40
11	C	Styrene	50	AcOH	5	95	35
12	A	Styrene	50	ACN	8	92	30
13	C	styrene	rt	AcOH	0	0	0
14	B	styrene	rt	AcOH	traces	99	20
15	B (48 hours)	styrene	rt	AcOH	traces	99	100 (45)

There is a very important detail that must not be overlooked. The reaction with styrene at high temperature in method B gave, as a product, a dimer whose presence can be confirmed by the integration in the ^{1}HNMR spectra, and, of course, of the MS data. Its presence was identified in some other conditions, however, not in this case. Because we did not successfully isolated any of the products we decided to synthesize one of them in a clean way so we could later compare the results. It was also found a very unusual side product (A, scheme 15) that was isolated in a substantial amount. The proposed mechanism for the formation of this enal is quite interesting and its formation has already been reported in literature[58].



Scheme 15 – Proposed mechanism for the formation of the enal in the reaction of Styrene in condition B at 115ºC.

By analyzing the table we can make some interesting assumptions, which corroborate our initial hypothesis, namely that there is an increase of the [1, 5] H-shift when high temperatures are employed. If we take the condition B into consideration, which, arguably, has the harshest conditions (TMDAM + H_2SO_4) from the three, we can recognize that the reaction does occur at lower temperatures (entry 15); however, a longer reaction time must be used. The other conditions at this exact temperature (entries 13, 14) failed completely to produce any of the expected products.

There seems to be an almost linear relationship between the temperatures and, in this case, the amount of products, even though the ebullition point of the solvent must also be taken into account (entry 4). After these results we can safely assume that in order to have the highest amount of crude quantity we must use the highest temperature possible, always taking into consideration the boiling point of any compound in the reaction, either reagent, reactant or product.

Most of the examples are related to styrene; however, the results with undecene are also along the same line and corroborate the thesis that in AcOH there is an increase in unsaturated

tertiary amine when lower temperatures are used (entry 9). This fact could be observed in the initial studies when the repetition of the literature results was made.

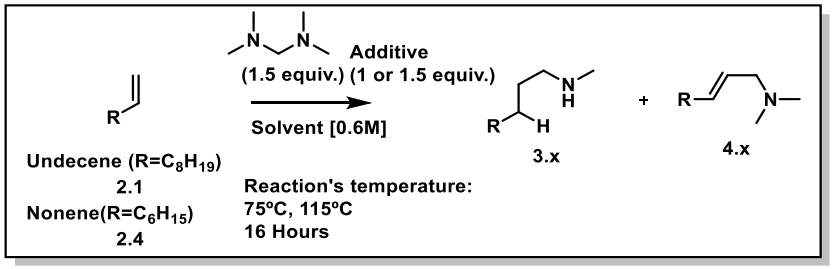
As discussed in the introduction, the most popular way to form amines is by reductive amination. We decided to react the corresponding aldehyde and methyl amine in a reductive amination which yielded, unexpectedly, the isolated dimer. After this, we can safely conclude that we had in fact also synthesized the dimer in the aminomethylation of styrene. There are, logically, some ways around this problem, which we will be focusing on later. Nevertheless, at this point in time, we decided to concentrate on the optimization and possibly amplification of the experimental results.

Because of the formation of the dimer that derived from the use of styrene we decided to use only aliphatic alkenes in following studies, even though they have yielded lower quantities of product.

II.1.4- Study on the effect of additives

The optimization process has to be focused not to waste resources and time in useless reactions, so our first idea involved the study of a possible range of additives that could enhance the reaction. We also knew that would be quite difficult not only because there is almost no literature about this topic but also because making alkenes more nucleophilic is still unexplored. For that reason the major role of the additives is to enhance the reactivity of the iminium specie.

The most common catalysts when alkenes are used to activate them in an electrophilic manner, suggesting complexation and/or protonation using Browsted or Lewis acids. Notwithstanding, we decided to explore these additives mainly because they probably would interact if the iminium specie as well.



Scheme 16 – Study on the effect of an additive on the reaction of aminomethylation. ^{1}HNMR yield was calculated using 1,3,5 trimetoxybenzene as an internal standard.

Table 5 -Additive optimization (substitution of H_2SO_4). All reactions were conducted in a 1 mmol scale. Acid Base extraction was used. [a]Average was used when more than experimental result was obtained .[b]Solution was quenched with 50% NaOH and extracted with diethyl ether. TMDAM – Tetramethyldiaminomethane.

Entry	Electrophilic species + X equiv. of Additive	Olefin	Temperature [ºC]	Solvent	4.x (%)	3.x (%)	Crude quantity mg (^{1}HNMR yield, %)
1	[a]TMDAM+ 1.5equiv.HBF_4	Undecene	115	AcOH	0	99	100 (20)
2	TMDAM + 1 equiv. HBF_4	Undecene	115	AcOH	0	99	75 (10)
3	TMDAM+1.5equiv. HCl	Undecene	115	AcOH	0	99	70 (10)
4	TMDAM+ 1equiv. HCl	Undecene	115	AcOH	0	99	70 (8)
5	[a]TMDAM	Undecene	115	AcOH	0	99	165 (38)
6	TMDAM + 1.5equiv. TfOH	Undecene	115	AcOH	-	99	205 (20)
7	*TMDAM + 1equiv. TfOH	Undecene	115	AcOH	-	99	160 (10)
8	TMDAM +1.5equiv. H_2SO_4	Undecene	70	TFA	-	-	225 (10)
9	TMDAM + Tf_2NH	Undecene	70	AcOH	-	-	100 (50 % conversion)[b]
10	TMDAM + 1.5equiv. Tf_2NH	Undecene	70	AcOH	-	-	100 (30 % conversion)[b]
11	TMDAM+1.5equiv. H_3PO_4	Undecene	115	AcOH	-	99	210 (40)
12	TMDAM + 1.5 equiv. HBF_4	Nonene	115	AcOH			40
13	TMDAM + 1.5equiv. HBF_4	nonene	115	AcOH			50
14	TMDAM + 1.5 equiv. TsOH	nonene	75	ACN	-	-	40

To our dismay, none of the newly tried conditions were able to increase the yield of the reaction. When H_3PO_4 was employed (entry 11), the crude amount and ^{1}HNMR yield were fairly close to the ones that we could achieve in our best condition. This made sense because in literature both acids (H_2SO_4 and H_3PO_4) were found to have very similar results[54] both in isolated yield and relative quantity of amine products.

One experiment that was very important for the comprehension of this intricate reaction was the reaction without the sulfuric acid: with only the AcOH to promote the formation of the iminium specie from the TMDAM. This rehearsal (entry 5) lead to a lower amount of product and subsequent crude quantity; however, the point is that the reaction worked anyway and because of this we can state that the acidic medium must be very important for the product to be formed. The role of the sulfuric acid was still unsure, possibly it can promote at a faster rate the formation of the reactive iminium ion from the TMDAM and it leads to a general increase in yield.

As a replacement for the very aggressive additive H_2SO_4, several tries were conducted with tetrafluoroboric acid (HBF_4, entry 1). The thought process behind it involved different reasons. Up to this moment we were positive that we needed a strong acid to promote the reaction and, as mentioned before, we needed the alkene to be nucleophilic and not to react with anything in the medium but the iminium ion. Tetrafluoroborate is also known as a non-coordinating anion and usually only coordinates to strongly electrophilic metals centers [59].We also assumed that, if it were to bind, it should be only slightly with the charged iminium specie that is more electrophilic than the alkene, leading for a more susceptible attack of the alkene . For that reason it seemed like a good option to try. Because of the previous characteristics of the tetrafluoroborate anion we repeated the reaction a couple of times with another aliphatic alkene (entries 12, 13) with no improvement.

The next acid that was attempted was the very commonly used hydrochloridric acid (HCl): it also had very poor results; this can be attributed to side reactions that can occur (entry 3/4). One explanation could be that a 37% solution of HCl was used, which led to high quantities of water in the medium, this acid is also known for the hydroclorination of alkenes [60]. Despite the fact that usually these types of reactions are very slow, we still decided not to pursue similar reaction conditions such as gaseous HCl or other halogen acids.

Given that up to this moment the best example is when sulfuric acid was used, which, in comparison with all examples so far is also considered one of the strongest acids, we decided to

explore other additive that have an even higher dissociation constant. An example is the trifluorumethanesulfonic acid (TfOH) that besides being less susceptible to oxidation/reduction is also less likely to lead to sulfonation of the alkene, which is a good point[61]. All this consideration led to lackluster, albeit interesting results.(entries 6,7) Despite the yield being lower, we could see that the spectra were less "clean" with a higher amount of side products that could be seen as uncharacterized peaks in both ^{1}HNMR and Mass Spectra. This can be explained by the high reactivity of an extremely strong acid than can lead to even more side reactions, and even degradation.

Furthermore, a nitrogen-based super acid was also experimented: bis(triflyl)imide (Tf_2NH), whose similarities to TfOH are evident and had a similar outcome to its relative. In its experiment we actually found a good amount of starting material (50% - entry 9 and 10) than can be partially attributed to the lower temperature that had to be used due to its boiling point (70º C). One other important fact is that the imide is actually a stronger acid than TfOH but still no full conversion was achieved [62].

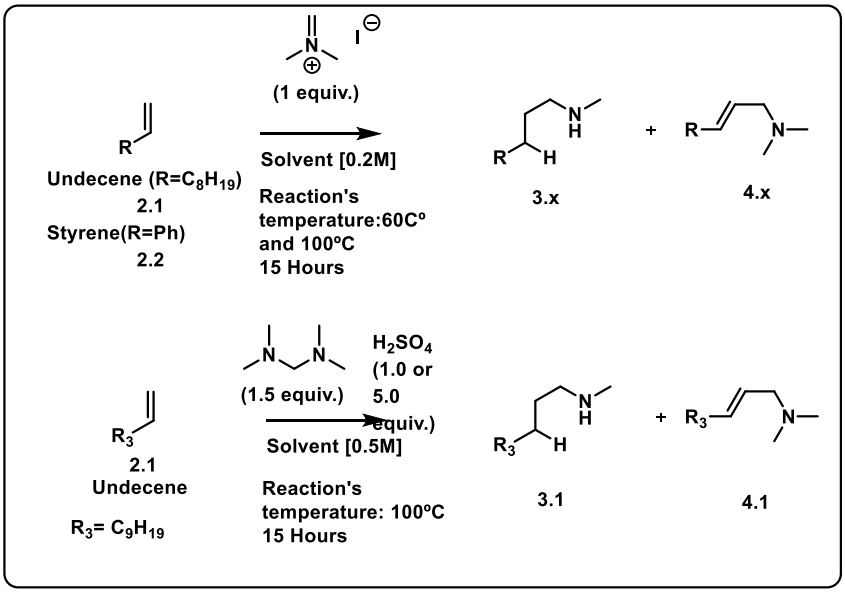
We even attempted the use of trifluoromethanesulfonic acid with a polar aprotic solvent such as acetonitrile in place of acetic acid. This is also one the perks in using this type of brownsted acid catalysis (solubility in polar solvents)in comparison to strong mineral acids that lead to decomposition and side reaction with the solvents. Nevertheless, no desired products was identified (entry 14)[63].The synthetic utility of these brownsted acids cannot be understated especially in the early development of C-C bond formation using them as catalysts. However, their importance in the catalysis of aminomethylation was found to be diminute.

The last but still very important result is one that is quite different from all others. One experiment was the use of another acid as a solvent instead of AcOH: trifluroacetic acid. In this case, we continued to use sulfuric acid as an additive. The results were, at a first glance, quite interesting, mostly because of the high amount of crude quantity of recovered material that was superior to our best result so far. Similar to the case of TfOH, there appeared to be a large amount of side products from the analysis of the obtained spectral data. In retrospective, this was the most important so far and the reason for that will be discussed in depth further down the report.

One last remark about the optimization of the additive is that no catalytic amount was used (only 1 and 1.5 equiv.) because, after much consideration, there was no possible regeneration of the catalyst during the reaction.

II.1.5- Study of the effect on the solvent in Aminomethylation

The substitution of acetic acid for an alternative source of acid meant that we had to scope other solvent in order to test if we could improve the reaction conditions. For that reason we devised a scope with solvents that ranged between polar and non-polar. In order to have similar reaction conditions, most reactions were conducted at 100ºC.



Scheme 17 - Study on the solvent scope on the aminomethylation reaction. [1]HNMR yield was calculated using 1, 3,5 trimetoxybenzene as an internal standard.

Table 6 - Solvent scope. All reactions were conducted in a 1mmol scale and in [0.2M]. Reactions were quenched with NaOH (50%) and extracted with diethyl ether.[a] Solvent concentration of [0.66M]

Entry	Electrophilic species	Olefin	Temperature [Cº]	Solvent	4.x (%)	3.x (%)	Crude quantity mg ([1]HNMR conversion,%)
1	Eschenmoser's salt	Styrene	rt	d_8 toluene	-	-	-
2	Eschenmoser's salt	Styrene	60	DCE	20	80	40
3	[a]TMDAM + 1.5equiv. H_2SO_4	Undecene	100	$MeNO_2$	-	-	223 (0)
4	[a]TMDAM + 1.5equiv. H_2SO_4	Undecene	100	DMSO	-	-	200 (20)
5	[a]TMDAM + 1.5equiv. H_2SO_4	Undecene	100	ACN	-	-	158 (50)
6	[a]TMDAM + 1.5equiv. H_2SO_4	Undecene	100	Dioxane	-	-	150 (0)

7	[a]TMDAM + 1.5equiv. H_2SO_4	Undecene	100	Cyclohexane	-	-	205 (20)
8	[a]TMDAM + 5 equiv. H_2SO_4	Undecene	100	ACN	-	-	80 (Decomp)
9	[a]TMDAM + 5 equiv. H_2SO_4	Undecene	100	Dioxane	-	-	250 (Decomp)
10	Eschenmoser's salt	Nonene	100	$MeNO_2$	-	-	50
11	Eschenmoser's salt	Nonene	100	cyclohexane	-	-	180 (20)
12	Eschenmoser's salt	Nonene	100	DMF	-	-	140 (60)
13	Eschenmoser's salt	Nonene	100	Isopropanol	-	-	180 (100)

A very important observation is that large amounts of product were not being identified because they were not in the final organic phase. One of the methods we used to identify all the components in the reaction was to do the reaction itself in a solvent can allow direct ^{1}HNMR analyses for the characterization (i.e. deuterated solvents). An experiment we did not perform so far was the reaction with a non-polar solvent (for example toluene) and the difference it made in the product composition. Following these two facts, we decided to experiment the reaction in deuterated toluene (entry 1 – d^8 toluene). The results showed the presence of olefin in large quantities but side products were identified.

A quick glimpse at the table suggests that most of the conditions that were tested were unsuccessful in improving the method. Though we were not able to achieve an improvement, still most experimental data are precious because we can still gather a large amount of information from it.

That being said, the only experiment in which some product was identified was the reaction with 1, 2-dicloroethane (DCE) and eschenmoser's salt (entry 2). The conversion was complete, however, a low amount of crude led to the rejection of this condition. The dipolar moment of the 1, 2-dicholoroethane is lower than the acetonitrile's and, for that reason, a larger quantity of unsaturated amine was to be expected due to the favored elimination reaction – this possibility was confirmed by the experimental results.

In contrast to apolar solvents that were discussed so far, various polar solvent were used instead of acetic acid in conjuction with TMDAM. Sulfuric acid was continued to be used as an additive in order to be able to compare to previously obtained results. Another reason is that the sulfuric acid should catalyze the formation of the iminium specie more easily – this should be especially hard for a polar solvent such as nitromethane ($MeNO_2$,) or dimethylsulfoxide (DMSO,) to catalyze promote by itself. It should be noted that the latter solvent led to very low conversion but to high amount of crude quantities that suggest the presence of unwanted products (entries 3 and 4).

This very trend could be seen when less polar solvents were used such as dioxane and cyclohexane (entries 6, 7). Another common fact in these results was the color of the solution, that instead of a yellowish pale solution had a very dark orange crude. One possible rationalization would be that the 1.5 equiv. of sulfuric acid were enough to generate the iminium specie and for that reason side reactions occurred meanwhile. To try to understand this possibility, larger amounts of sulfuric acid were employed (5 equivalents) In both experiments (entries 8 and 9) a similar outcome was possible to be underlined: a decomposition of the solution and all its components seemed to have occurred. Much like in previous reactions where larger quantities of acid were used the spectral data seemed to suggest a large plethora of compounds. This result complements the fact that a higher amount of acid does not translate to higher amount of desired product.

When acetonitrile was used, there was a considerable amount of crude material. However, the conversion was lower than expected with this method (50 % - entry 5). Another fact that is common to all the previous attempts is that no product could be easily identified from the crude ^{1}HNMR.

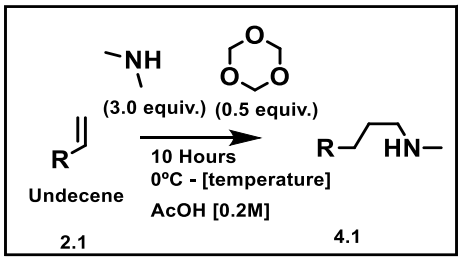
When talking about the scope of the solvent in condition A, the results obtained did not have a clear improvement compared to the original method. The use of polar solvents such as $MeNO_2$ and DMF (entries 10 and 12) still had lower conversions and crude material recovery.

A surprising result was the use of isopropanol as a solvent for the reaction. This was the first example of alcohol as a solvent that we used so far, and the reason for that is quite evident. If a free alcohol was to be used, it could attack the electrophilic iminium salt preventing the reaction from happening. No starting material was found, yet, the expected product was not identified either.

After this battery of results we can conclude that an important factor such as the acidity in the medium is crucial for the outcome of the reaction. We can also say that the amount of additive is significant, because of high quantities of acid leading to decomposition.

II.1.6 - Alternative methods for the formation of the iminium species

So far, from the all the conditions, the one that seemed to be most promising was the reaction with TMDAM in acidic medium. Because of this, we wanted to explore other conditions that could shed some light on some of the intricacy associated with the method.



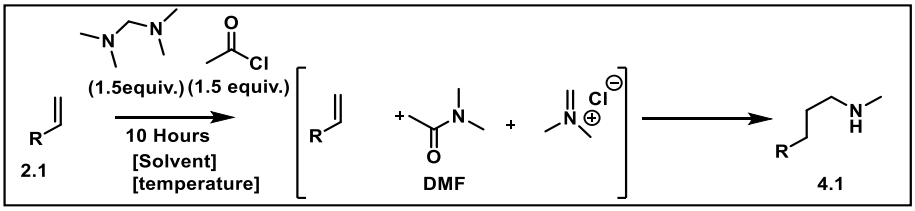
Scheme 18 – Reaction of aminomethylation using the iminium specie generated *in situ*. Reaction was conducted in a 1mmol scale of undecene. ^{1}HNMR yield was calculated using 1,3,5 trimetoxy benzene as an internal standard.

The first alternative method was using a "masked" version of formaldehyde – trioxane – and dimethyl amine to generate the iminium specie *in situ*. In order for this reaction to occur, we decided to use acetic acid to promote it. This method had already been used for this type of reaction[54] but was almost immediately substituted by TMDAM which led to less reactants to be added to the solution and a higher overall yield.

Table 7 –Generation of the iminium species using dimethylamine and trioxane. All reaction were conducted in a 1mmol scale and in [0.2M] .Reactions were quenched with NaOH (50%) and extracted with diethyl ether.

Entry	Temperature [°C]	^{1}HNMR yield (%)
1	115	18
2	75	10
3	rt	0

All the results from this experiment fall in line with our previous conclusions about the effect of temperature in aminomethylation . In this reaction conditions, even at high temperatures the yield was very low compared to the direct use of TMDAM in the reaction. To explain this outcome we can state that, in order to generate the iminium, water must be eliminated and this may significantly diminish the amount of iminium I due to hydrolysis.



Scheme 19 – Reaction of aminomethyation using the iminium specie generated *in situ*. Transformation was conducted in a 1mmol scale of undecene.

The second and last variation that we explored as far as different methods are concerned is the generation *in situ* of a chloride iminium salt very similar to the Eschenmoser's salt. The thought behind this experiment is that we could elucidate the role of the acid in the reaction. Another important factor is that we could understand if the iminium was being formed by identifying the presence of one of its side products – DMF – that could be seen by ^{1}HNMR, for example. One last advantage would be the use of milder conditions with neutral pH and organic solvents (DCM or ACN for example).

Table 8 – Generation of the iminium specie in situ without any acid - All reaction were conducted in a 1mmol scale and in [0.2M] .Reactions were quenched with NaOH (50%) and extracted with diethyl ether.

Entry	Solvent [0.2M]	Temperature [ºC]	Conversion (%)
1	DCM	0	20
2	ACN	0	25
3	DCM	40	50
4	ACN	40	50

According to the analysis of all the results from this reaction, no product was possible to be seen in the ^{1}HNMR spectra and for that reason only conversion is represented on the table. This fact, *per se*, already clarifies that the reaction did not to occur. As mentioned before, we know for a fact that the iminum was generated due to the presence of DMF in the ^{1}HNMR spectra. Much like in the previous reaction, the increase in temperature led to a higher conversion, however, in this case, no product was found.

We can say that these experiments proved useful anyway, thanks to them showing how the acid medium is not necessary only to promote the formation of the iminium ion but to the successful proceeding of the reaction itself.

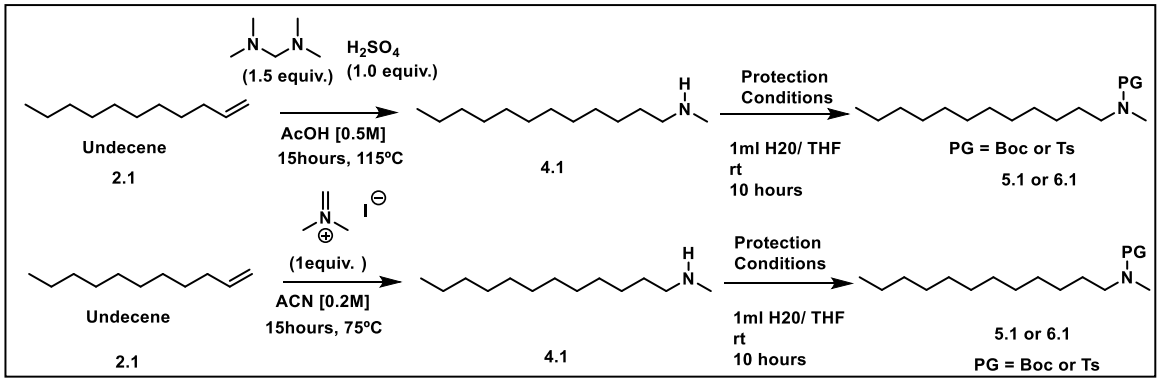
In a quick summary of all the work so far, we could establish three major problems that we still had to solve. The first one, and the one we decided to tackle thus far, was that the yield

was considered quite low, and in our best result, using TMDAM/ H_2SO_4 in acetic acid at 115ºC we only had 50% of amine. The second problem was one that we knew existed from the beginning and also consists in one of the major limitations of the method – the reaction only worked on terminal alkenes or pseudo activated alkenes (cyclic alkenes)[54]. The last one, and whose urgency was quite evident at the time, is that until this point we did not have any isolated product mostly because of the nature of the substrates we employed. We attempted several types of chromatography (silica, alumina and RP18) with different eluent compositions and packing conditions (NH_3, NEt_3 during the packing), whose intent was to diminish the interaction between the free amine and the stationary phase that could lead to diminished yield. However, despite all this effort, we could not effectively isolate our secondary amines.

II.1.7- Study of the protection of a secondary amine

One possible solution for this hardship was the functionalization of the amine to something that would be more easily isolated and identified. The most common solution is to go around the reactive and troublesome N-H bond by protecting the amine moiety. There were several possibilities to get to the protected amine. The more common methods, which are also easy to execute, are for example using Boc_2O or Tosyl chloride to generate the carbamate and sulfonamide respectively [64].We came to the conclusion that both of these moieties had the potential stability and ease in characterization that would be helpful to fulfill this task.

All attempts were made from a portion of the same reaction, this meaning that they all derive from the same initial reaction (entries 1 -7 and 1-12). This was to eliminate possible errors during the repetition of reaction (for example in weighting reagents, etc.)



Scheme 20 – Reaction's scheme of aminomethylation followed by protection using Boc Anhydride or Tosyl Chloride . Olefin was used in a 1mmol scale. ^{1}HNMR yield was calculated using 1,3,5 trimetoxybenzene as an internal standard.

Table 9 – Study on the protection reaction with a secondary amine. All reaction occurred in a 1mmol scale. WU – Work up procedure

Entry	Reaction Conditions	Protecting agent	Protection conditions	WU	Isolated Yield (%)
1	TMDAM	$(Boc)_2O$ (5equiv.)	NaOH Until pH14	None	10
2	TMDAM	TsCl (10 equiv.)	NaOH until pH 14	None	48
3	TMDAM	$(Boc)_2O$ (3 equiv.)	$NaHCO_3$ (3 equiv.)	Evaporation	-
4	TMDAM	Boc_2O (3 equiv.)	Et_3N1.5 equiv. in THF	yes	7
5	TMDAM	Boc_2O (3equiv.)	DMAP (10%) in THF	yes	23
6	TMDAM	Boc_2O (3equiv.)	DMAP (10%) Et_3N 1.5 equiv. in THF	yes	20
7	TMDAM	Boc_2O (3equiv.)	Sulfamic Acid in 1 mL THF	yes	53
8	TMDAM	Boc_2O (3 equiv.)	Sulfamic Acid in 1mL H_2O	yes	60
9	Eschenmoser's Salt	TsCl (5 equiv.)	NaOH until pH 14	None	7
10	Eschenmoser's Salt	Boc_2O (3 equiv.)	$NaHCO_3$ (3 equiv.)	Evaporation	8
11	Eschenmoser's Salt	Boc_2O (3 equiv.)	Et_3N 1.5equiv. in THF	yes	7
12	Eschenmoser's Salt	Boc_2O (3 equiv.)	DMAP (10%) in THF	yes	14
13	Eschenmoser's salt	Boc_2O (3 equiv.)	Sulfamic Acid (10%)in 1mL H_2O	yes	26

Before we started analyzing the results, we were aware that we needed to establish a purification procedure to be able to isolate the protected amine. Both in the case of the tosylated amine and Boc-protected amine we tried several types of chromatography techniques (alumina / silica / RP18), the one which worked better was when we used silica flash chromatography. Despite this, we encountered several problems, the main one being Boc_2O contamination because of the excess that is employed in the reaction and the non-polarity character that our compounds exhibit. Because of this, we had to use very apolar eluents (heptane /diethyl ether – 0%- 2%) to be able to separate the mixture. We also used a large amount of silica in a long column to improve the separation.

We thought about several strategies to protect the amine that, at the same time, have the least impact in the yield of the reaction. It is known that a portion of the compound is lost during transfer between flask and even during extractions. To prevent this, we tried to do the protection directly after the reaction was terminated. There were two distinct possibilities to achieve it.

One of them is by the addition of the protection agent directly to the crude mixture; in this case, it should also be accompanied by a base due to the fact that most protection procedures that involve amines require deprotonation of the ammonium salt. This meant that we had to make the medium alkaline. This was done by adding NaOH in excess so that the pH of the solution was near 14 (entries 1, 2 and 8). This method led to some very positive results, especially when tosyl chloride was used as a protecting reagent. When boc anhydride was used in this procedure the yield was far lower, most likely due to its high reactivity to all the components in solution in comparison to the tosyl counterpart.

The second method that bypasses the use of work up or transfer of any kind is by simple evaporation of the solvent. Problems can emerge from this procedure, especially in the case of the reaction using TMDAM, which would need very low pressures in order to evaporate AcOH, and, when low boiling point-alkenes are used, even the expected product itself can evaporate. In this case, the best you can achieve is the hydrosulfate ammonium salt due to the presence of sulfuric acid.

After all the solvent was evaporated, $NaHCO_3$ was used to promote the boc protection (entries 3 and 9). There are various examples of boc protection in literature and we attempted to experiment most of them[65]. Here, the protection was not that successful with only 8% of isolated yield in the reaction with the eschenmoser's salt.

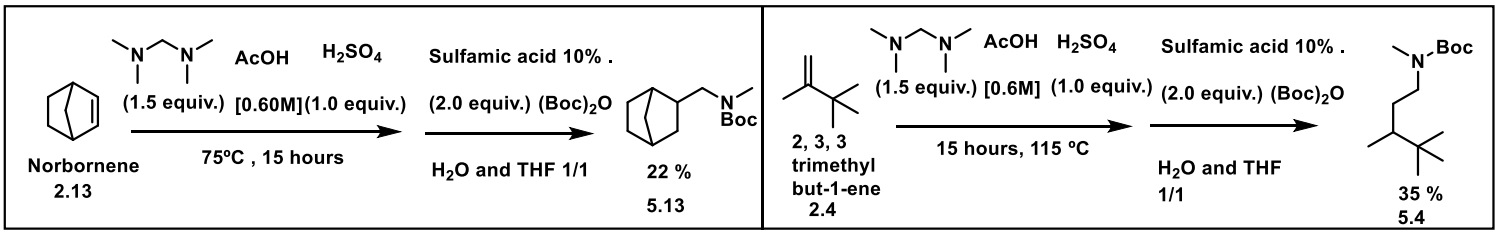
As mentioned before, boc protection can be done in several different ways and some of the most common reagents are, for example, Et_3N and DMAP, which can be used at the same time or only one of the two in conjunction with the boc anhydride. In regard to the results, they were better than most but not the best so far. The reaction with DMAP had a larger amount of isolated products than the reaction with NEt_3 (entries 4, 5 and 10, 11). An interesting result was that the use of NEt_3 and DMAP had a lower yield than DMAP usage alone (entry 12). It is noteworthy that a work up has been done in these last cases, which means that some product could be lost during the process.

In our pursuit to find a better way to the protection we searched for a method that could be feasible in acidic medium due to the characteristics of the previous reaction. As a matter of fact, we did not encounter many methods that satisfied those conditions, but one: we found an alternative method using an uncommon reagent to catalyze the reaction. Catalytic amounts of sulfamic acid were reported to protect amines in very mild conditions and for that reason it seemed the best option to try [66]. It is clear that in terms of results this method had the best outcome of all (entries 7 and 8). The original method was performed in a neat fashion, solventless; however, we experimented on THF and H_2O as solvents due to solubility issues. As far as the solvent is concerned, water had the best results with 60% of isolated yield (entry 8). Another advantage that we encountered was that we could evaporate the solvent and do the column directly without any loss in the extraction procedure.

One important factor that is necessary to comment on is that there is a slight discrepancy between the isolated yield and the ^{1}HNMR yield from previous results. In the case of the use of TMDAM, we found that we had a higher yield than it was expected, which is, obviously, good news. The use of internal standard as a method to quantify reactions has some problems associated with it, mainly related to reproducibility aspects because of the purity of the internal standard [67]. In spite of being a well establish procedure for routine analysis, errors can occur. Another comment that can be made is that having isolated yields is always better for obvious reasons – it accurately measures the wanted product of the reaction. ^{1}HNMR yield usually is very usefull because it gives an estimation of the yield on a certain point of the reaction.

II.1.8- Initial scope of alkenes

Now that we had a complete procedure that involves a protection in very mild, accessible conditions (air atmosphere), we started to focus on the resolution of another issue associated with the method thus far. We need to enlarge the scope of the method by adding a couple of examples to test its generality. One on them was a cyclic olefin, another one a terminal olefin with different characteristics. Two of the substrates that we were very interested in experimenting on were norbornene and 2, 3, 3-trimethyl-but-1-ene (2.4). One explanation for this fact is that knowing that the reaction follows a cationic intermediate, possible carbon esqueleton rearrangements can occur especially at high temperatures (115ºC) in acidic medium. Norbornene(2.13) is quite geometrically constrained and in 2, 3, 3-trimethylbut-1-ene case a migration of a methyl group is possible.



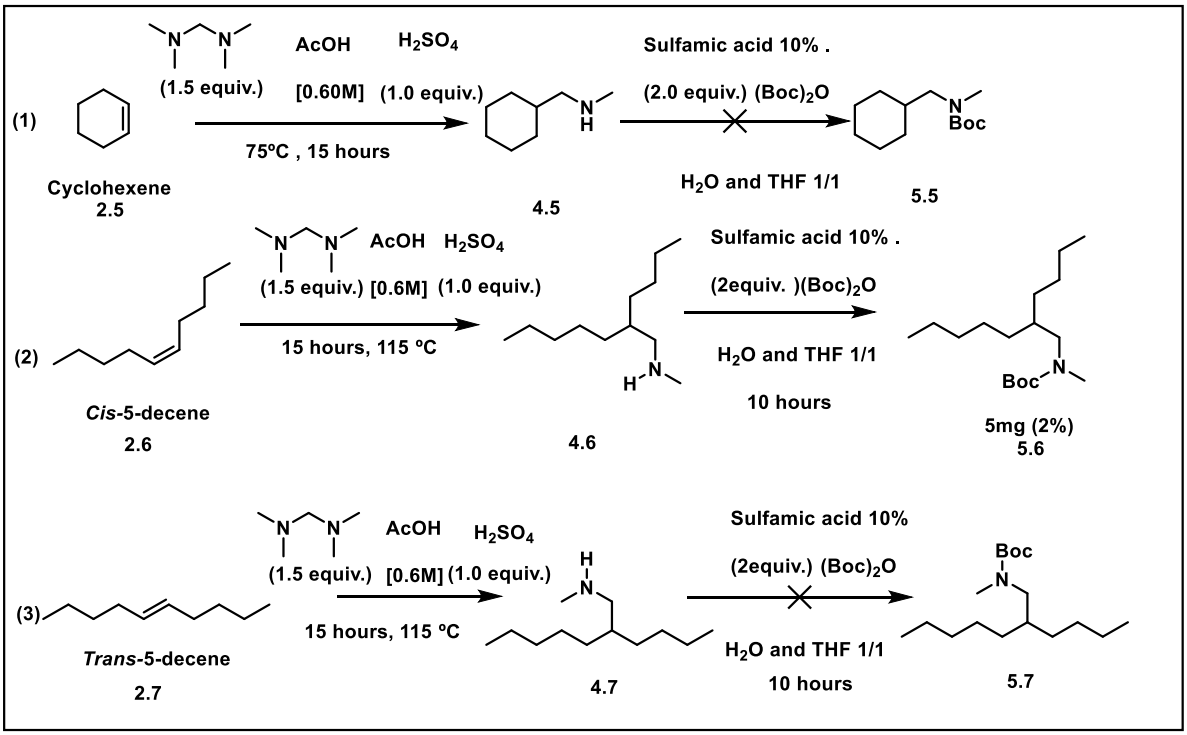
Scheme 21 – Aminomethylation using 2,3,3-trimethyl-but-1-ene and norbornene as a starting olefins. Reaction were conducted in a 1mmol scale. In the protection reaction, 3 equivalents of Boc_2O were used in [0.5M] of H_20 and THF. Work up consisted of extraction using diethyl ether.

The reaction using these two examples of highly reactive alkenes was motivating. The reaction yielded, in both cases, the expected product. The conversion was complete in both cases and the purification was done with ease just like the previous compounds in this method.

Although at a first glance these do not seem like the best results, they were still quite motivating because even though we used somewhat harsh conditions we were able to isolate amines from supposedly sensitive substrates. We also managed to do the reaction with an internal olefin, norbornene, most likely because it should be more reactive due to geometrical constrains. In the previous two reactions we were not able to isolate any product of Wagner/Meerwein type rearrangement[68].

The next step after this initial study would be to confirm if the method does in fact work with more simple internal olefins or not. Three examples were chosen: Cyclohexene(2.5), *cis*-5-decene(2.6) and *trans*-5-decene(2.7). Cyclohexene was chosen because it would be possible for us to compare the results with our previous one (norbornene) that had, theoretically, more acti-

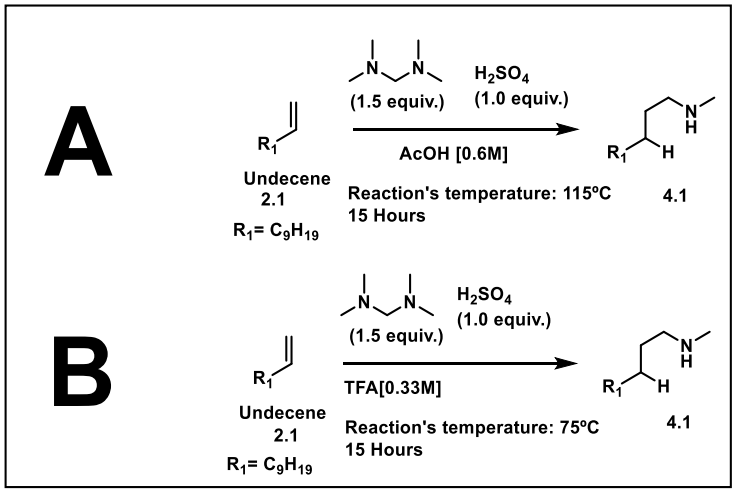
vated cyclic olefins. The reason in using the decene isomers was that we could both study the importance of geometrical isomerism and have another example of internal olefin.



Scheme 22 – Initial scope using internal olefins. (1) - cyclohexene; (2) – *cis*-5-decene; (3) *trans*-5-decene

The experimental data confirmed that internal olefin without any factor of activation would not be reactive enough to attack the iminium ion. These experiments were conducted in duplicate but still no favorable results were achieved.

At this point we had to go back to the roots of the work done so far in order to surpass this hurdle. By drawing comparison between different information we acquired in different parts of this journey we arrived to a very interesting notion: the attempt in which we used TFA instead of acetic acid to activate the TMDAM. At the time, we assumed that the reaction generated a lot of secondary products due to the "messy" spectral data collected. Another situation where we could see the same pattern was in the scope for the solvent where we used H_2SO_4 in excess. A conclusion can be made that probably, the additive, sulfuric acid can induce an over acidic medium when used in conjunction with TFA.



Scheme 23 – Reaction A – Undecene (1mmol) with 1.5 equiv. of TMDAM and 1.0 H_2SO_4(1.0 equiv.) in [0.66M] AcOH at 115ºC. Reaction B – Undecene (1mmol) with 1.5equiv. of TMDAM and 1.0 equiv. H_2SO_4(1.0 equiv.) in [0.66M] TFA at 75ºC. Reactions were quenched with 50% NaOH and extracted with diethyl ether.

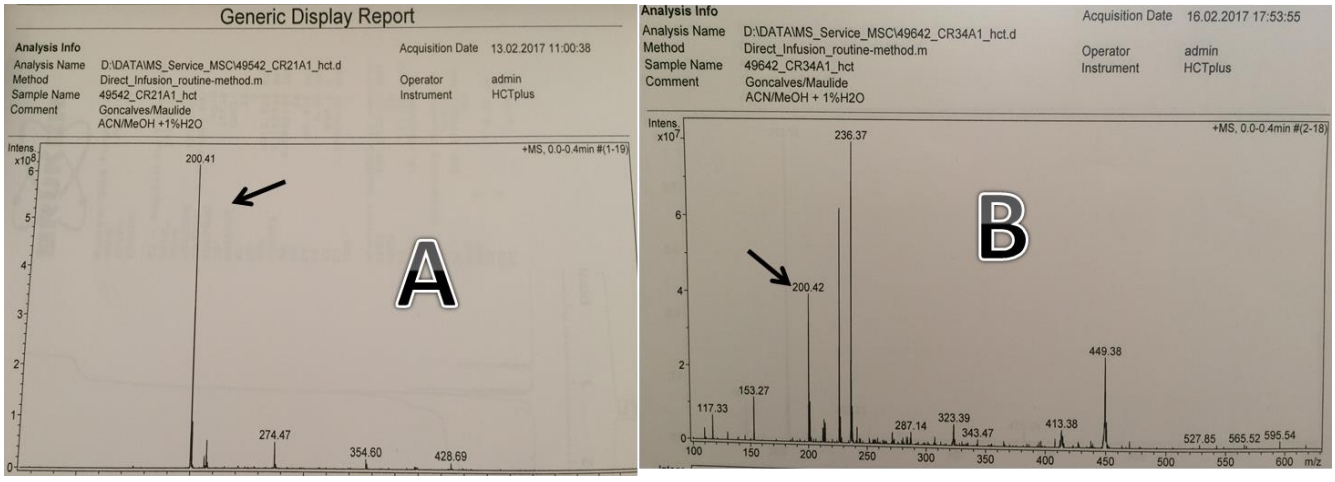
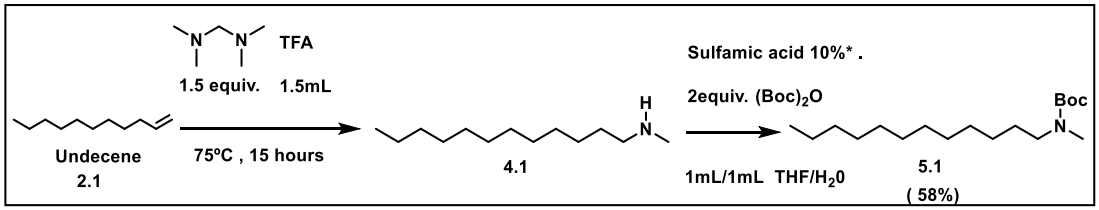


Figure 1 – A – Mass spectra of the reaction with undecene and TMDAM with AcOH/ H_2SO_4 at 115ºC. B- Mass spectra of the reaction of undecene and TMDAM with TFA/ H_2SO_4 at 75ºC.

By comparing these mass spectra (A and B) we can observe that there are major differences between them. The only common peak is the one from the expected product (200.41 – black arrow) all the rest of the spectra has a different profile entirely. The effect of the H_2SO_4 in excess is clearly shown in the contrast between both experimental data. A reasonable step after this enlightenment was to repeat the reaction without any H_2SO_4. Another advantage associated with these reactions is that it uses a significantly lower temperature (75ºC).



Scheme 24 – Reaction of undecene and TMDAM in TFA at 75ªC followed by protection using sulfamic acid (10%) and Boc_2O (2 equiv.).

After this preliminary and very successful study, we moved to the enlargement of the scope to confirm if it indeed solved any problem, and maybe, following the same tendency, if it increased the overall yield of the reaction. In order to be able to compare it with previous results, we decided not to deviate from prior substrates.

Table 10 – Initial scope of previous used substrates with TMDAM (1.5 equiv..) and TFA[0.66M] at 75ºC. Protection using sulfamic acid and Boc_2O(2 equiv.) followed by evaporation and flash silica chormatography (heptane/ diethyl ether(0-2%)). Isolated yeild was calculated after two steps. [a] reaction conditions were 1.5equiv. of TMDAM + 1.0equiv. of H_2SO_4 in [0.66M] of Acetic Acid at 115ºC.

Entry	Olefin (2.x)	Isolated yield (%)	Previous yield(%)[a]
1	Undecene (2.1)	58	53
2	Nonene (2.3)	60	55
3	2,3,3-trimethyl-but-1-ene (2.4)	36	30
4	Norbornene (2.12)	35	22
5	Cyclohexene (2.5)	5	0
6	*Cis* 5 decene (2.6)	35	2
7	*Trans* 5 decene (2.7)	30	0

By looking at the isolated yield of the amine we can see that we have accomplished a breakthrough in terms of surpassing the limitations of the method. The reaction with alyphatic alkenes (entries 1 and 2) showed a higher yield that, despite not being a huge increase, has now acceptable yields. We can achieve a much bigger growth in the case of norbornene whose isolated yield went from 20% to 35% (entry 4), and, to a less extent with 2,3,3-trimethyl-but-1-ene(entry 3). These latter results are not considered the best; nevertheless, an important fact must be taken into account: that at this point most reaction conditions (solvent concentration, TMDAM quantity, etc..) have not yet been optimized.

The last three entries of the table were subjected to great scrutiny since these substrates did not work with our previous conditions. By looking at the reaction with cyclohexene (entry 5), we can say that this internal olefin did work though in very low yield. While with both isomers of decene there was a substantial amount of secondary amine that was isolated, and, thence we can say that the method does work with internal olefins. The very slight difference between both isomers, at this point, is not enough to rationalize one preferential isomer instead of the other; in any case, *cis*-5-decene should provide a higher amount of product due to a more convenient nucleophilic attack. One could also infer, almost in a farfetched way that, the *cis* vs *trans* destabilization of the olefin could also make the *cis* olefin more reactive – this could in fact but true , however it should not be as illustrative at low temperatures such is the case of this reaction. We would like to reiterate that there can be a considerable increase in overall yield when the method is sufficiently optimized.

II.1.9- Optimization of the solvent used in the extraction

In most methods there exists a part of the work up that is systematically overlooked because its impact is thought to be negligible – we are talking about the extraction solvent. In this case, before the protection reaction, we noted that our compounds could interact with the organic phase mostly because of the free amine moiety that appeared after the work up. We decided then to scope the extraction procedure. Similarly to the optimization of the protection reaction, we used the same initial reaction and divided it in different portions to diminish potential experimental error.

Table 11 – Optimization of the extraction procedure. [a]Relative polarity towards water/ values taken from Christian Reichardt, Solvents and Solvent Effects in Organic Chemistry, Wiley-VCH Publishers, 3rd ed., 2003.

Entry	Solvent	Relative crude (%)	Relative Polarity[a]
1	Chloroform	100	0.259
2	Diethyl ether	80	0.280
3	Ethyl Acetate	85	0.228
4	Dichloromethane	70	0.309
5	Heptane	45	0.012

To facilitate the analysis of the results we calculated the relative percentage of crude in regard to the highest result, which allows for a faster analysis and deduction of the experimental data. From the outcome of this experiment we can see a tendency between the amount of compounds that are extracted from the aqueous phase and relative polarity of the solvent. It does not

come as a surprise that the more polar the solvent the higher the amount of compounds are dissolved by it; however, in this case that assumption is not totality proven. The polar compounds are more easily dissolved in polar solvents because there is interactions between both dipoles generating enough energy to dissolve the molecule[69] .

There is quite a big difference between the relative polarity of heptane and the others. Here, its poor ability to be used as an extraction solvent does not come as a surprise. Because the difference between the other examples is so small, other types of explanation can be made to justify the experimental results. There is a possibility for secondary interactions that can lead to higher solvatation in chloroform instead of the others.

II.10.-Optimization of the solvent concentration

Still on the topic of solvents, there is a very important factor that must be taken into consideration in every reaction that is performed. Solvent concentration can have, and has, in many cases, a crucial role in the pathway of the reaction [70].The yield of a reaction can sometimes also be easily modulated by this factor as well.

Table 12 – Optimization of the solvent concentration in the reaction of TMDAM (1.5 equiv.) and TFA at 75ºC. Undecene was used in 1mmol.

Entry	Solvent Concentration [M]	Isolated yield(%)
1	2	63
2	1	59
3	0.66	57
4	0.2	40

There seems to be a correlation between the concentration and the overall yield of the reaction. There are some possible explanations for the phenomena. One of them is directly related to the definition of concentration itself. If it is said that a solution is more concentrated than the other, it simply means that one solution has more solute per area than the other. In a reaction this fact merely indicates that, spatially, the molecules are closer to each other and, for that reason, they have a higher chance for productive interaction *per* time, leading to an increased reactivity.

One of the important objectives when a methodology is being developed is that it has to be easily reproducible. This being said, the focus is not always on the yield; other factors such as the presence of side products and scalability) must also be carefully measured. In this case, even though the best results are at lower concentrations, we found that several byproducts were

identified and for that reason we decided to use an intermediate concentration (entry 3) that, so far, allowed us to have a cleaner purification of the products.

II.1.11- Optimization of the TMDAM equivalents

The next step of optimization is logically the optimization of the equivalents of TMDAM, which is directly related to the number of iminium specie that are in the reaction medium. Up to this point, the only consistent side product that was isolated was the dimer of the expected product. The quantity of TMDAM can also help limit the formation of the unwanted product because the probability of the product encountering another olefin would be lower if a higher amount of iminium specie were in the medium.

Table 13 – Optimization of TMDAM amount used during the reaction between the olefin 1 mmol and TMDAM in TFA [0.66M] at 75ºC

Entry	Olefin	TDMAM (equiv.)	Isolated yield (%)
1	Undecene	0.9	42
2	Undecene	1.5	60
3	Undecene	3	65
4	Undecene	4.5	71
5	Styrene	1.5	73 (with dimer)
6	Styrene	4.5	62

A number of different equivalents of TMDAM were tested in order to test its effect on the yield of the reaction. When it was used as a limiting reagent (entry 1) it did in fact lower the yield by a substantial amount. Since the diamino compounds are in fact cheap and easily accessible, they can be used in high amounts without a huge impact on the methodology. The best results were attained when 4.5 equivalents of TMDAM (entry 4) were employed (entry 4) and, thus, we decided to continue using this amount in the upcoming reactions.

The previous assertions about the possibility that an excess of TMDAM would inhibit the dimer formation were substantiated by the last two entries on the table: using styrene the trend was the same. Despite the decrease in yield there is no doubt that when 4.5 equivalents of diamine were used no dimer was formed in the process.

The second entry in the table, the original conditions, is the one that was employed in the scope of the reaction, so a theoretical increase in yield is possible in some case where the outcome is not satisfactory enough.

II.1.12-Study of possible modifications in the formation of the iminium using TMDAM

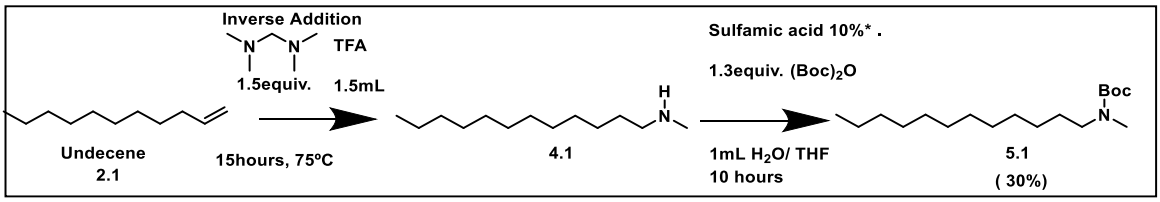
In regard to the method itself there are still a number of different directions that could be taken to further develop the method. Still on the topic of the importance of TMDAM in the reaction, there are two distinct possibilities that could be interesting to test out. Up to this point the TFA is added to TMDAM in an ice bath slowly over 5-10 minutes before the olefin is added. There are reports that the formation iminium salt could take more time than that despite the fact that in those examples no strong acid is used to promote its formation [71].In any case, the experiments were done to put the theory to a test.

Table 14 – Optimization of the time for the formation of the iminium. Reaction consisted on TMDAM (1.5equiv.) and TFA [0.66M] with undecene (1mmol) at 75ºC. Work up and protected as were referred in the method.

Entry	Time [minutes]	Isolated yield (%)
1	15	65
2	30	45
3	60	35

It is evident from the analysis of the results that a longer time is not necessary for the formation of the iminium especie. The acid medium must be efficient enough in generating the iminium in the first moments. The decrease of yield in the longer times can be related to side reactions that consume the iminium specie in solution.

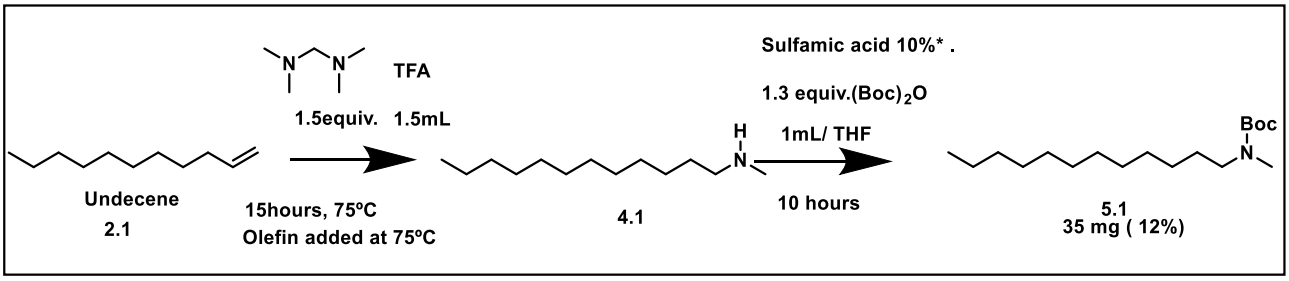
The other experiment still related to this early part of the reaction is the inverse addition of the initial reagents. The addition of the TMDAM to the TFA was experimented because the formation and stability of the iminium ion formed upon they reaction can be influenced by the relative concentration in solution and the other counterion being present at the time. The order of addition of reagents and reactants can often play a key role in the outcome of the reaction.



Scheme 25 – Reaction of aminomethlation with inverse addition of the reagents. TMDAM was added after the TFA. Reaction was performed in a 1mmol scale.

There was a significant lowering in terms of the amount of isolated yield after the inverse addition of the reagents. The addition of TFA is usually done very slowly because of the intense fuming that comes from its use. Experimentally speaking, the solution fumed way more in comparison to the original procedure when the olefin was added after the TFA and TMDAM.

In relation to the addition of olefin, another experimental condition that we thought about and tried was the addition of the alkene at 75ºC when the TFA was in ebullition. The idea behind this is that the reaction at lower temperatures does not occur, which means that there is a time in-between when the reaction is not actually proceeding.



Scheme 26 – Reaction of aminomethylation with addition of the olefin at 75ºC. Reaction was performed in a 1mmol scale.

The outcome of the previously represented reaction did not come as a big surprise mostly because of the problems with additions at reflux. It is actually common for this kind of addition to have a lower yield, adding the olefin to a very acid solution at high temperatures can lead to decomposition and other types of reactions besides aminomethylation.

II.1.13-Scope using the optimized method for aminomethylation

Now that we successfully optimized the method we decided to experiment it with different type olefins. We decided to use a very large scope containing diverse functional groups in order to test if the method is applicable to a wide range of substrates.

In our preliminary tests using other olefins we came to a standstill regarding purification: the problem was that our current method, flash chromatography using heptanes as eluent, was not able to separate efficiently all products. After experimenting with different conditions we decided to use toluene as the main component of the eluent. With this unusual solvent we were able to separate all compounds and the more polar ones using a very small percentage of diethyl ether (0-2%) to modulate eventually the polarity in the column.

While being a very small modification to the method, in the work up of the purification reaction instead direct evaporation we used a diethyl ether extraction to obtain the crude for the flash chromatography. The reasoning behind this was that some of the compound in the scope can be volatile and to make sure everything was obtained in the same conditions, we altered the procedure.

To facilitate the comprehension and analysis of our purposed scope we decided to categorize it in 5 different groups, which are:

1 – Linear aliphatic olefins

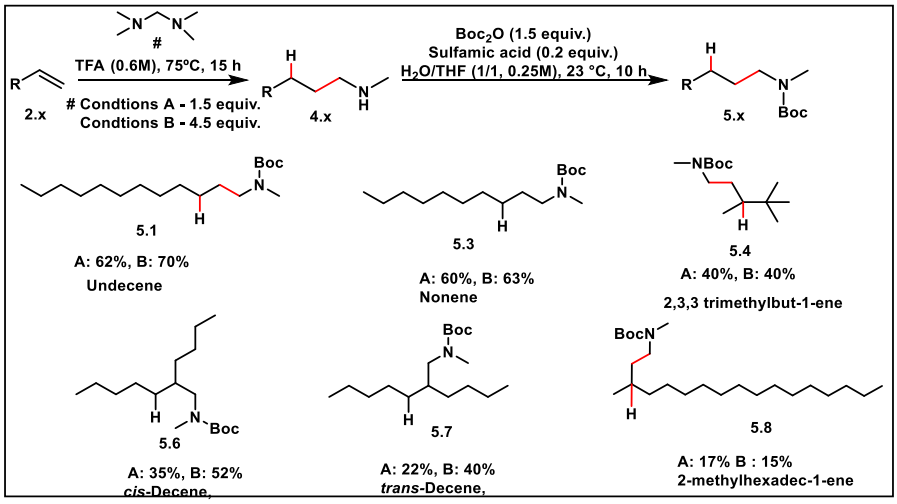
2 – Cyclic olefins

3 – Olefins containing other functional groups

4 – Alkynes

5 – Dienes

The first group is composed by aliphatic olefins which are not activated in any way. We used as examples terminal, internal and 1, 1 disubstituted alkenes.



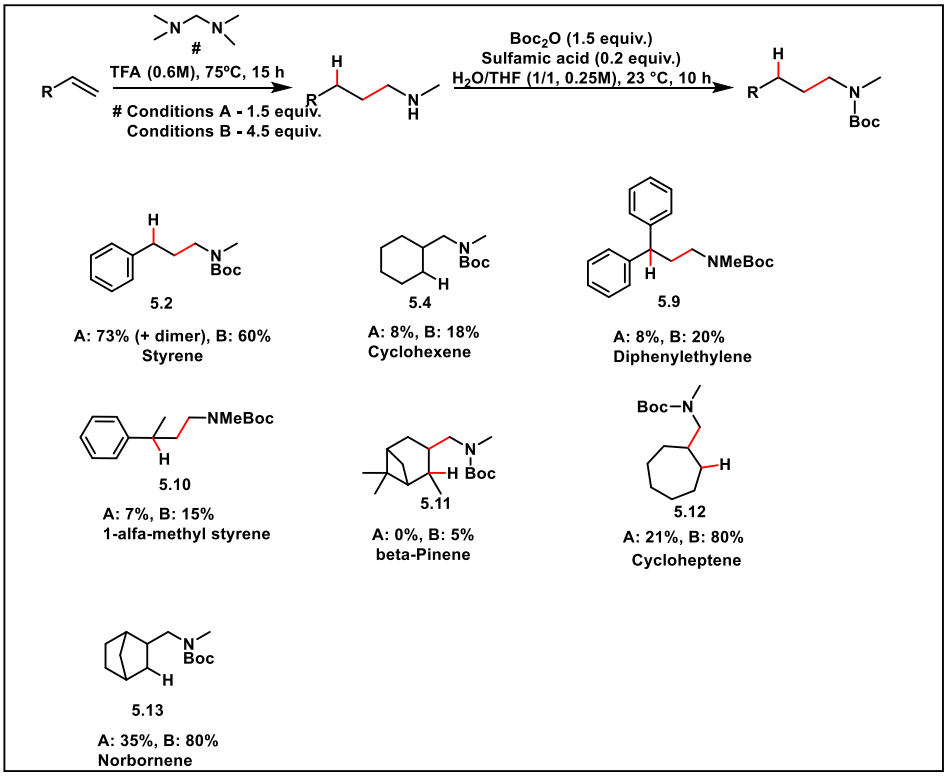
Scheme 27 – Group 1 – Scope of aliphatic alkenes using the established method.

It comes as no surprise that the best results were attained when terminal alkenes were used (5.1, 5.2 and 5.4). We theorize that the reasoning behind it should be related to the stereochemical availability of these substrates, which, in part, is substantiated by the results of the internal olefins, which are less successful. The comparison between the 5.6 and 5.7 (geometrical isomers) also correlates to the geometry, having a role in the reaction with the *cis* isomer lead-

ing to higher quantities of amine. The poor yield on the 1, 1 disubstitued alkenes (5.8) is not easily explained because they are more stericly available than the other examples of internal olefins.

In terms of the importance in the equivalents of TMDAM (condition A vs B), from the experimental data, the *cis* and *trans* isomers have the most discrepancy between values, although for all the substrates a higher amount of diamino leads to a higher yield.

The following group includes a high variety of examples of olefins containing either a cyclic moiety in their skeleton or an aromatic one. This is also the group with the most substrates, mostly because they are easy to use and have a large importance in synthetic chemistry.

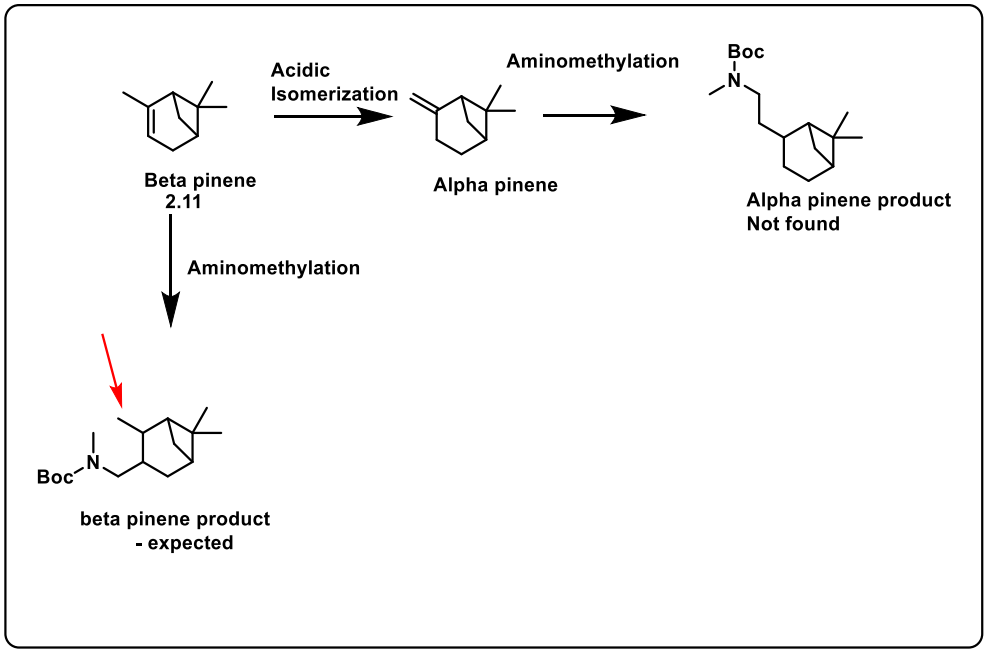


Scheme 28 – Group 2 – Scope of cyclic containing alkenes using the established method.

In this compilation we find results that range between the best and worst in relation to the isolated yield throughout the whole work. A clear tendency can be seen if we take the constrained olefins into account (5.12 and 5.13) that have, altogether, the best results in this methodology.

The aromatic olefins have, generally, disappointing results with only styrene being an exception. If we compare the three aromatic compounds we can see once again a trend regarding steric hindrance. This being said, the difference between diphenylethylene (5.9) and 1-alpha methylstyrene (5.10) should be greater in order for this effect to be more elucidative.

From this scheme, the most reactive substrate is without a doubt beta-pinene (5.11), which is known to isomerize easily in acidic medium. A small quantity was isolated when a higher amount of TMDAM (condition B) was used; this trend in increased yield with increasing the amount of TMDAM can be seen even in this category of substrates. Because the ^{1}HNMR spectrum was not obtained in the best condition we were not sure if isomerization to alpha pinene occurred.

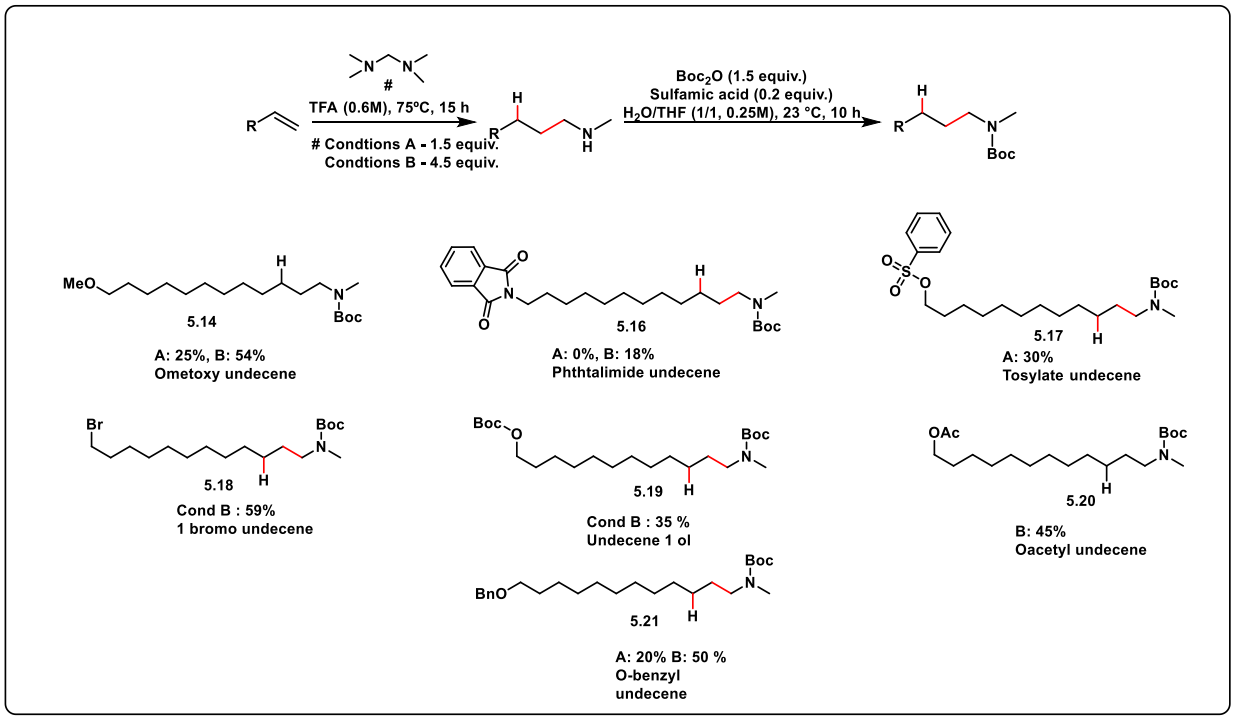


Scheme 29 – Representation of the isomerization of Beta-pinene to alpha-pinene and its corresponding aminomethylation product.

If alpha-pinene did exist in the medium the product would be essentially different .The methyl group from the expected product was easily identifiable and no similarities from the supposed spectra of the alpha-pinene product were found.

Cyclohexene (5.4) has been, from the beginning, one the most difficult substrates to work with due to the constant low yields. The optimization of the method undoubtedly helped increase the overall yield using this substrate.

This next group is arguably the most important. The reason behind it is that it establishes the limits within the method itself, meaning that the procedure utility is directly associated with the functional groups tolerance it has.



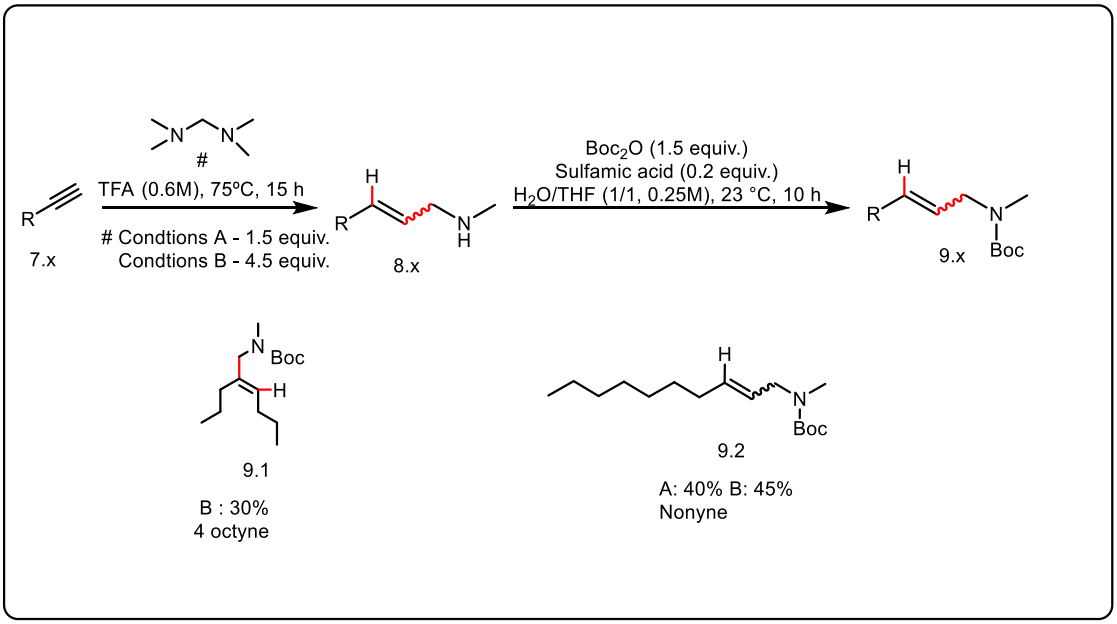
Scheme 30 – Group 3 – Scope of functional groups tolerance using the established method.

The results associated with this part of the scope were received with much enthusiasm because, we were concerned about the harshness of the method, it could be very limited in terms of its ability to tolerate more sensitive moieties. In general, however, most results mimic the ones from the original aliphatic skeleton, which is a very good sign. Two substrates that we would like to highlight are the free alcohol and the ester (5.21 and 5.20, respectively) due to them opening a realm of possibilities that were not expected in the beginning.

In this group there are a lot of substrates where the condition A was not tried out because condition B proved to be the most rewarding from the point of view of the yield and the excess of TMDAM as discussed earlier is not such a drawback.

The following examples resulted from an extension of method to alkynes. In the initial experiment we did not expect the same type of product, an allylic amine, because, alkynes are less nucleophlic than alkenes and for that reason the reaction should be possible but more difficult. The electrophilic substitution leads to a formation of a vinylic carbocation in the case of alkynes that is less stable than the carbocation formed when alkenes are employed. The reason

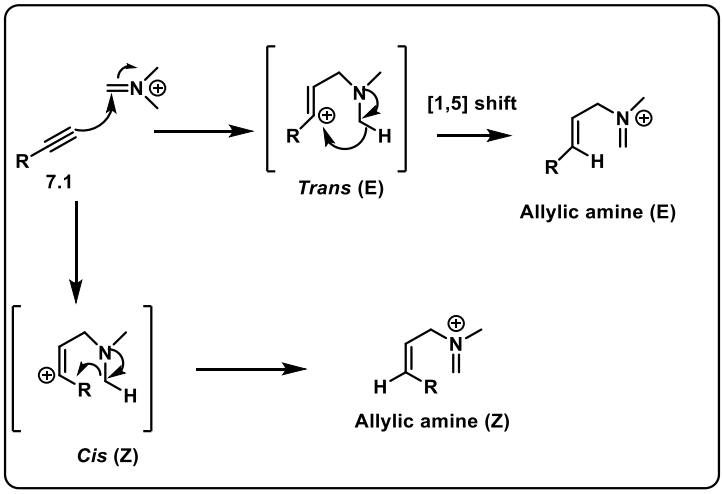
for that is that the cation is located on the sp2 hybridized orbital which requires more energy to be formed.



Scheme 31 – Group 4 – Scope of alkynes (7.x) using the established method.

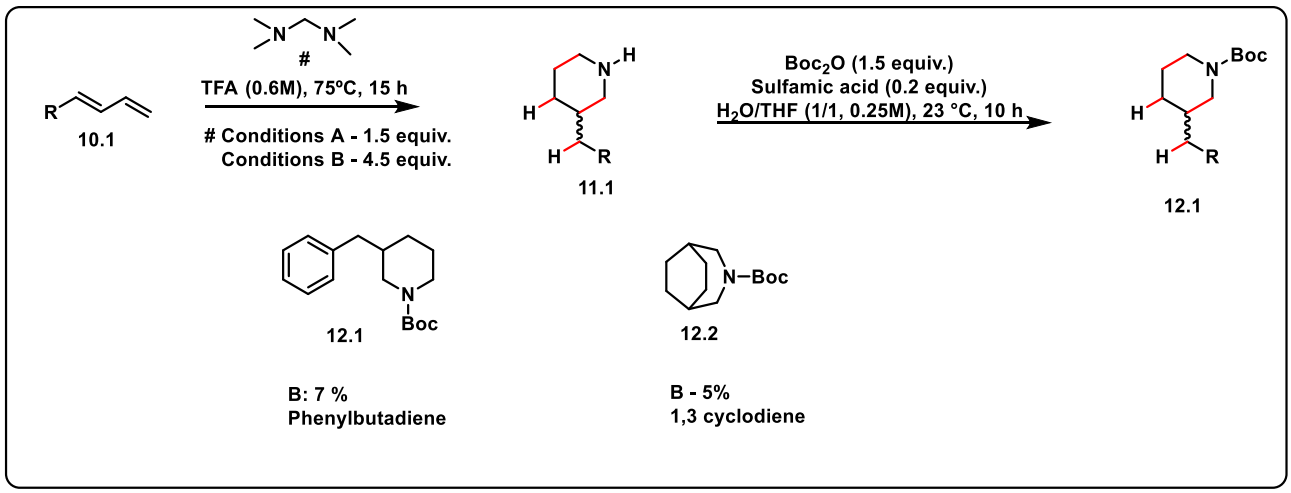
Despite not being the highest yielding substrate of the method, it was overall very successful in accessing an allylic amine. This very interesting moiety is quite difficult to synthesize especially directly, as when using this method. Much like in the rest of the method the internal alkyne (9.1) achieved lower yield than the terminal (9.2) one, even though only by a slight amount. If we compare the reaction with the alkene correspondents we see that the difference in yield between the two alkynes is less evident, which makes sense because of the sp hybridation that confers a linear disposition. This can also be an explanation for the surprisingly good yield of the reaction. Because the alkyne is linear the steric interferences are suppressed in comparison to the alkynes.

When employing alkynes to form allylic amines a new possibility arises in the products: two geometrical isomers could potentially be generated. From the analysis of ^{1}HNMR and ^{13}CNMR we can state that the *trans (E)* isomer exists in higher amount, however, we cannot undoubtedly confirm that the *cis (Z)* was not formed as well. From the proposed mechanism the *trans (E)* isomer should be formed preferentially because the hydrogen shift should occur in a *syn* fashion.



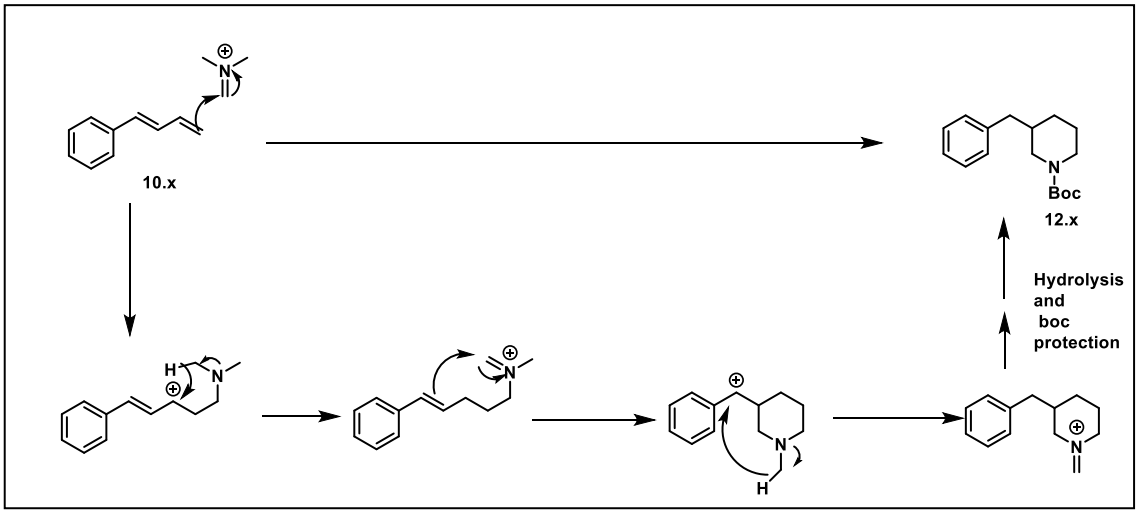
Scheme 32 – Proposed mechanism for the formation of an allylic amine by aminomethylation.

The last item on the list of the categorized groups belongs to the dienes. The expectations behind this group of alkenes is quite low owing to the fact that they are intrinsically unactivated as a nucleophile.This problematic can be so dramatic that the reaction can simply not work at all.



Scheme 33 – Group 5 – Scope of dienes using the established method.

The immediate perception of this transformation is that it is capable of generating very interesting moiety in a single transformation. From the analysis of both products, the reaction must occur stepwise involving two different nucleophilic attacks and, subsequently, two [1, 5] hydrogen shifts. One could also make an argument about the formation of an allylic carbocation that would stabilize the transition state leading to a lower activation barrier that would suggested a high likelihood that this transformation would occur.

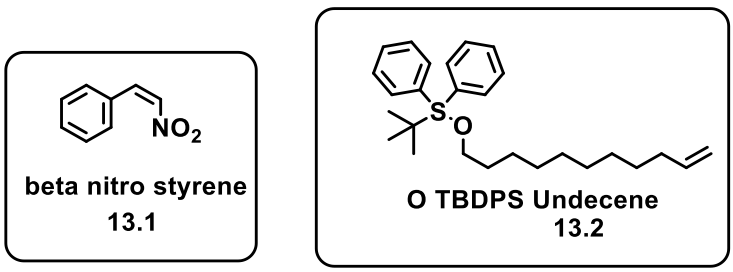


Scheme 34 – Proposed mechanism for the formation of the cyclic aliphatic amine using phenylbutadiene.

The low isolated yield of the transformation is challenging testimony of the complexity of the reaction, as referred before, so they are not completely unexpected. One possible explanation for this problem is related to the amount of intramolecular transformations that have to occur to reach the final product – consequently, there are more possibilities for a misstep to happen in the process.

II.1.13-Substrates that did not work: limitations of the method

One of the most important factors when studying a methodology, along with its applicability, is its limitations. During the process of discovering the method many experiments are bound not to work properly due to certain factors. The relative importance of this experimental data is, equally preeminent to the positive data: in simpler words, all results are crucial for a better understanding of the method, whether they have high yield or do not work at all. Therefore, we decided to compile some substrates in which the reaction did not occur, classifying them as limitations for the method.



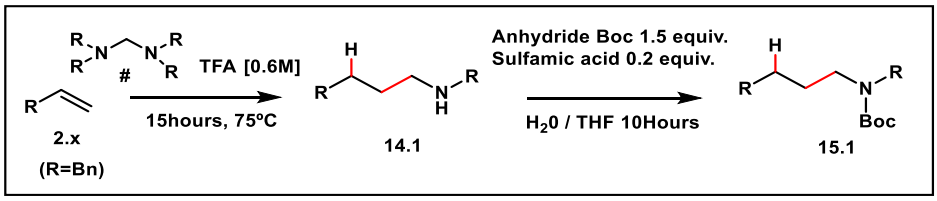
Scheme 35 – Substrates that did not underwent the reaction of aminomethylation.

The previous scheme clearly states two major limitations of the method. Firstly, we have an example of deactivated alkene with beta-nitrostyrene which was recovered after no reaction took place. This experiment only clarifies that a nucleophilic alkene is required in the reaction of aminomethylation.

Secondly, the high acidic medium of the reaction should prevent the use of acid sensible protection groups such as silyl ethers. To elucidate this fact we experimented with one of the silyl ether most resistant to acid conditions – *Tert*-butyldiphenylsilyl (TBDPS). The reasoning behind its resiliency comes from the fact that in order to hydrolyze the silyl ether the oxygen atom must be protonated first. When TBDPS is employed, the oxygen lone pair is shielded very efficiently by the large steric environment around it, so that protonation actually occurs on the aromatic first, leading to its tough cleavage[72] .

II.2-Use of diaminoderivatives in aminomethylation

If we take a closer look at the transformation at hand, there are still several possibilities left to explore. One of them is related to the reagent that was most discussed during the course of the work – Tetramethyldiaminomethane – TMDAM. If the method were to work with different tetraaminomethyl moieties the potential of the reaction would increase immensely. The group represented in the scheme as R could very well be useful for synthetic chemistry, such as the benzyl moiety.



Scheme 36 – Reaction using tetrabenzyldiaminomethane as a iminium specie. Reaction performed on a 1mmol scale using 1 equiv. of tetrebenzyldiaminomethane in [0.6M] TFA at 75ºC. (R= Bn).

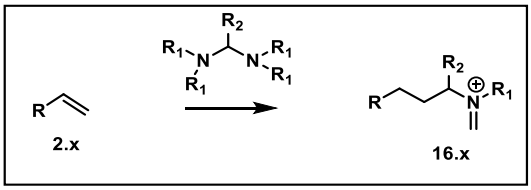
Taking into account the specific case of tetrabenzyldiaminomethane, whose synthesis is quite straightforward, we clearly see that there is a great deal of value that could come out of this transformation. When the reaction was attempted there was a huge setback in terms of purification. During the reaction dibenzylamine was also formed, leading to the formation of a by-product which is also protected as the Boc derivate during the second reaction. This side product proved really complex to separate from the main product by any mean.

Despite many different approaches in the purification procedure no significant advancement was made in the process of isolation of the product. Other different reactions were also tested in order to reduce the amount of side product that was generated. Perhaps the most logical one was using TMDAM as a limiting reagent that did not yield a very different result from previous experiments.

The outstanding value in this transformation is something that should not be underestimated – for that reason alternative methods should be studied in order to retrieve the desired product.

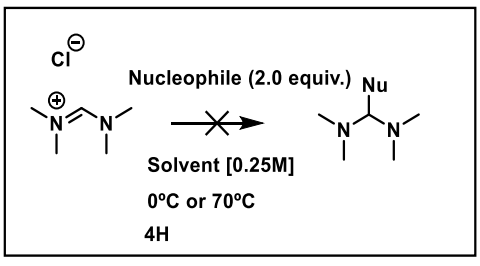
II.3-Synthesis of 1, 1-diamino derivatives

During the current work we scrutinized the TMDAM in many ways so that we could increase the yield of the reaction or insert a specific functional group on the molecule. If we take a closer look once again we can see that there is still another possibility for functionalization within this compound – substitution in-between both nitrogen atoms.



Scheme 37 – Aminomethylation reaction using 1, 1 disubstituted diaminoalkane.

The synthesis of 1, 1-diaminoalkanes is quite an unexplored area and that fact is elucidated by the low amount of symmetric 1,1-diaminoalkanes that are reported in literature. One example which we found that had a high potential was the use of an oxidized form of TMDAM: (Dimethylaminomethylene)dimethylammonium chloride and a nucleophile to generate the wanted product.



Scheme 38 – Formation of the 1 substituted tetramethyldiaminomethane using a nucleophile (2.0 equiv.) and 1mmol of iminium salt in [0.25M] of solvent. Addition of both reagents was done at 0ºC.

In this specific case a sodium salt of acetylene was used as a nucleophile [8].In theory, this transformation would allow us to insert any nucleophile in the desired position.

Table 15 – Reaction of (Dimethylaminomethylene)dimethylammonium chloride with the nucleophile (2.0equiv) in [╔0.25M] of the chosen solvent for 4 hours.

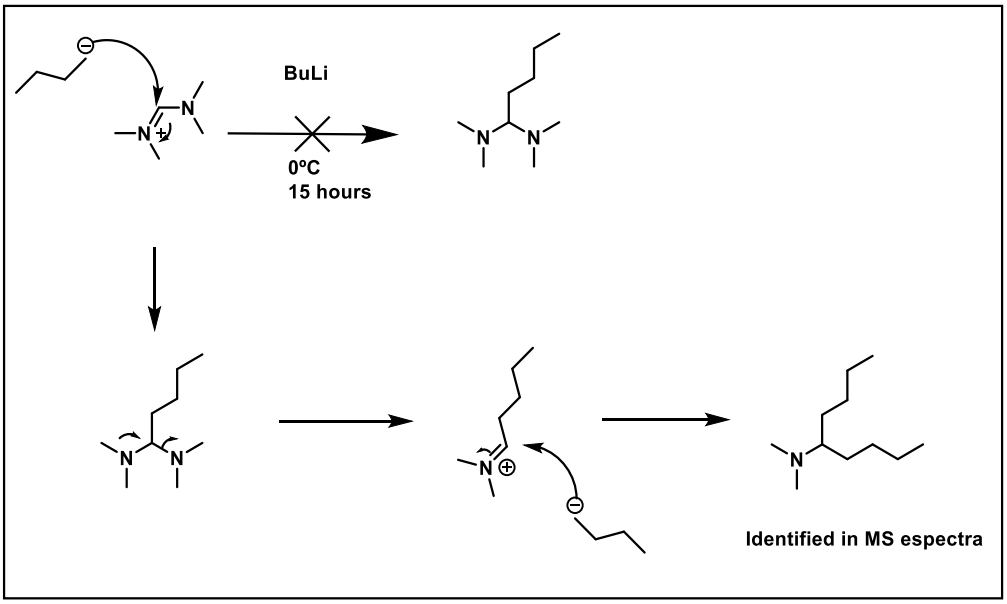
Entry	Nucleophile	Temperature (ºC)	Solvent
1	Butyl magnesium chloride	0	THF
2	Phenyl magnesium bromide	0	
3	Butyl magnesium chloride	70	
4	Phenyl magnesium bromide	70	
5	*n*-Butyl lithium	0	
6	*n*-Butyl lithium	0	DME

Using the method that was described before, it was not possible to synthesize the wanted product. In the first experiments we used low temperatures in order for the addition to occur in milder conditions because of the sensitivity of the product – for this same reason no acids/bases were used during the reaction. We also used two different Grignard reagents so we could test if the strength of the nucleophile was a limiting part (entry 1,2). The work up was similar in all cases and it involved the addition of NaCl followed by the extraction with diethylether. No compounds were identifiable from the crude ^{1}HNMR and HRMS.

In order to confirm that an addition was occurring higher temperatures were employed (entries 3 and 4). The only noticeable change was in the color of the solution that went from yellow to orange.

The next experiment involved a much stronger nucleophile: *n*-butyl lithium, which was used to make sure some addition, was occurring (entry 5). We also noted that *n*-butyl lithium could act as a base in the reaction, even though no evidence of that was found in the spectral data. As a last effort to increase nucleophilicity and lifetime of the nucleophile, the solvent DME was used because it is known to coordinate with lithium and increase the electron density on the carbanion, enhacing the addition[73].

The biggest hardship that we encountered in this part of the work was finding out which compounds were being created during the reaction. One suspicion of ours was that the whole extraction procedure was in some way preventing us from characterizing the products. The last attempt on this subject consisted in the addition of dry methanol to quench the reaction, and this allowed us to finally identify that over alkylation occurred.



Scheme 39 – Mechanism of the formation of the unwanted overalkylation product.

The unwanted product was identified by mass spectra and its formation can probably be inhibited by manipulating the experimental conditions.

III-Future Prospects

The vast potential of this transformation is also associated with the tremendous amount of work that should be done in order to explore it in the most exhaustive way.

In regards to the study of the method of aminomethylation that generates secondary methyl amines from TMDAM, the scope could still be enhanced. Our proposal involves the extension of the method to styrene-type derivatives that should prove to be very interesting substrates if the reactivity can be modulated to work with different substituents.

In terms of the functional group scope, there are some synthetically relevant examples that could be added, which include, among others, free amines, carboxylic acids and unprotected alcohols, to name a few.

A concept that was proven was that the reaction works with alkynes, which means that several substrates could be tried out in order to increase the range of usable materials, moreover an increase in the yield is desirable, yield which is only acceptable at this moment (40%).

An interesting study could be carried out, with competition experiments within the same molecule (internal olefin and terminal olefin) that would allow us to gather more information about the chemoselectivity within alkenes. Now that we talked about the necessity of increasing the scope of the method it is evident that the last parts of the work were also those that should be explored primarily due to the immense value that can be given to the method. We are mentioning the synthesis of other diamino compounds are the most important areas to study afterwards.

Second, and equally important, point to focus on for the developing of the work is the purification: when tetrabenzyldiaminomethane is employed this proves difficult, and the same situation could arise for similar substrates. At this point, even though the tetrabenzyldiaminomethane was the more interesting substrate, the reaction should be tried out with other derivatives to increase the methods scope.

The third point is the synthesis of tetraalkyldiaminomethane derivatives. Being able to access different derivates, whose purification can be done by distillation if high quantities are used in the reaction, would be a nice plus for the methodology.

The fourth and last topic that should be further investigated is the synthesis of the 1, 1-diaminoalkane derivatives. Despite the last developments, that showed that the dry methanol work up is necessary to effectively analyze the components of the reaction, there are other strategies that should be tried out first. It is also possible to synthesize these amines by condensation of amine and aldehydes. The purification should also be possible using this method. There are a few examples of this type of reactions and, to ease the purification procedure, an aromatic aldehyde should be used. Potentially, if the reaction with these iminium precursors does work, the amine product could contain an additional stereocenter. The diastereoselectivity of the process is certainly worthy of further study.

One common feature on the transformation that has not been properly mentioned in this work is that part of the originated diaminomethane compound is in fact lost due to hydrolysis. But at the same time the fact that the intermediate product before hydrolysis contains an iminium means that there is room for functionalization with nucleophiles or other reagents that would be worth exploring.

IV-Conclusions

It is safe to assume that the level of successfulness that we achieved in the study of this methodology was much higher than it was expected at first. The interest behind this transformation was the reasoning behind much of the effort that was put into this work.

One of the objectives that were set out during the writing of this report was that the positive aspects of this reaction would be highlighted throughout the work. The final methodology consists in a very simple transformation with commercially available and cheap reagents, in generally mild conditions. This latter phrase summarizes the importance of this work which also coincides with the characteristics that are pursued when a method is first studied. The straightforwardness of the reaction itself contrasts quite perfectly with all the problems that were described during the introduction of this work. The simplicity and accessibility of the reagents in comparison to other methods that generate the same final products is perhaps the best way to illustrate the developments achieved in this work; however, one cannot forget other factors that also compose this transformation.

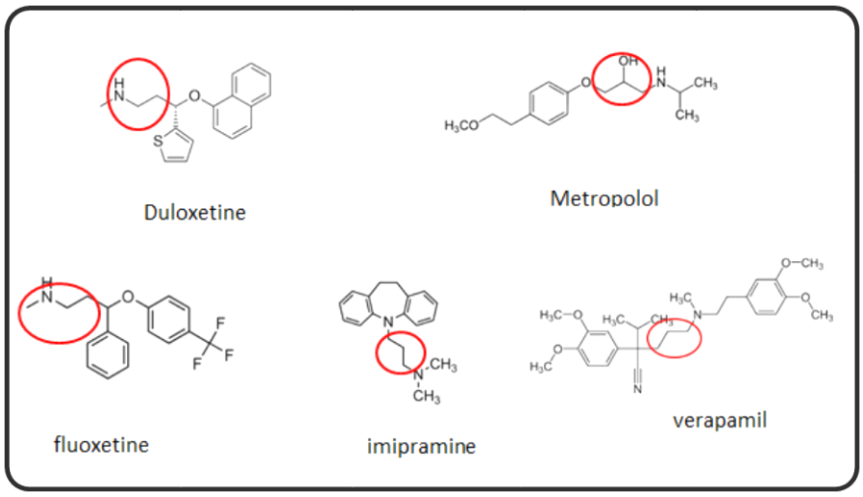
One of them is the self-explanatory atom economy which aminomethylation as a reaction perfectly exemplifies [74]. The complete involvement of both active reagents in this transformation speaks volumes about its value, particularly because it gives the reaction a certain level of greenness which is desired in the present times. Also a good point is scalability, which is inherently related to the usefulness of the reaction. In spite of no large scale attempt being made (1mol) to verify this fact, it should not inhibit it from working in large quantities. If we take the purification into account, distillation should be a perfect match if the reaction was to be adapted to an industrial scale. As a matter of fact, a possibility for commercialization of the method, in a form of a patent could be written.

Overall, the method did reach a large amount of compounds with good yield after a step of protection. This is relevant because it means that the yield could be fairly higher if the protection reaction does not occur in a quantitative manner. A balance was struck in a scope that included the most exemplificative substrates for an initial scope. A majority of the results point for this being a useful method that could be used in a number of synthetic applications.

We have also proven that we could access allylic amines directly through alkynes, something that, to the best of our knowledge, has not been demonstrated or reported in literature. Even if only a few examples were used with that type of substrates, they possess an im-

mense potential, particularly because the difference between terminal and internal alkynes did not show a clear decrease in the isolated yield. This fact is of the upmost importance because it proves that this transformation has a lot more room to be investigated. One could even assert that if the reaction does ignore the geometrical constraints that are supposed to exist in this type of reaction.

In the first paragraphs of this work we mentioned the importance of statistics in order to highlight the relevance of the amine group. Indeed, we can use once again the same statistics in a different manner - we pointed out that in the top 200 pharmaceutical products of 2016 we found that about 90% of the compounds had some kind of amine moiety in them. One very easy way to demonstrate the significance of a methodology is to show its possible transformation in the synthesis of some of these pharmaceutical products.



Scheme 40 – Examples of commercial pharmaceutical products that can be achieve by the application of the methodology[1].

The selected areas in these possible targets for the application of the methodology are the regions that would undergo the transformation of aminomethylation. These few examples bring light to the usefulness of this reaction in total synthesis of some compounds, which is only possible because of the functional group tolerance that is associated with it[1].

Another of the qualities that we searched for and found is that most functional groups remain intact during the reaction. There is still a large amount of functional groups to be tested, however, the method already showed resistance to very important moieties.

The fact that the iminium species are generated in situ by the use of others reagents is also a positive aspect since it allows for the functionalization of the electrophile. This would allow for the insertion of groups that have very sought for characteristics like the benzyl group.

The ability to modulate several parts of the amine moiety, which is inherent to this method, possibly increases the interest of the scientific community.

One other example of this is if a 1, 1-diamine derivative is used, which allows the formation of an amine with a high degree of substitution. As suggested before, it is possible that the reaction could generate only one diasteroisomer because of its nature. If it were to be true, it would increase to a larger extend the value of a reaction that would already be very valued.

Perhaps the most promising fact about the usefulness and desirability of this work is what is still left to do: the amount of possible pathways that can still be undertaken and explored corroborates and exacerbates its importance. This transformation is by no means perfect, however, there is still so much room to be developed and discovered that it makes it truly remarkable.

V-Experimental part

Disclaimer

Unless otherwise stated, all glassware was flame-dried before use and all reactions were performed under an atmosphere of argon. All solvents were distilled from appropriate drying agents prior to use. All reagents were used as received from commercial suppliers unless otherwise stated. Reaction progress was monitored by thin layer chromatography (TLC) performed on aluminium plates coated with silica gel F_{254} with 0.2 mm thickness. Chromatograms were visualized by fluorescence quenching with UV light at 254 nm or by staining using potassium permanganate/ Ninhydrin. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck and co.). Neat infra-red spectra were recorded using a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Wavenumbers (**νmax**) are reported in cm^{-1}. Mass spectra were obtained using a Finnigan MAT 8200 or (70 eV) or an Agilent 5973 (70 eV) spectrometer, using electrospray ionization (ESI). All ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AV-400 or AV-600 spectrometer at 300K. Chemical shifts were given in parts per million (ppm, δ), referenced to the solvent peak of $CDCl_3$, defined at δ = 7.26 ppm (^{1}H NMR) and δ = 77.16 (^{13}C NMR). Coupling constants are quoted in Hz (*J*). 1 and 13C splitting patterns were designated as singlet (s), doublet (d), triplet (t), quartet (q), sextet(sext), septet (sept). Splitting patterns that could not be interpreted or easily visualized were designated as multiplet (m) or broad (br).

The assignment of the compounds was done using 2DNM

V.1-Aminomethylation procedures

V.1.1-Optimized aminomethylation method

General procedure A

In a sealed tube under argon at 0°C tetramethyldiaminomethane (1.5 equiv.) was carefully dissolved in TFA [0.6 M] .After the addition of the desired olefin (1 equiv.), the tube was sealed and covered from visible light.

The sealed tube was heated to 75 °C for 15 hours before being quenched with a 1N aqueous solution of HCl (3 equiv.).After the resulting mixture was stirred 15 minutes, then an aqueous solution of 50 % NaOH was added dropwise until the pH became alkaline (pH 14). The solution was extracted with chloroform (4x) and the combined organic fractions were washed with brine and dried over potassium carbonate.

After evaporation at reduced pressure of the volatiles, sulfamic acid was added directly to the crude (0.5 equiv.). Distilled H_2O [1 M] and THF 1 [M] were, finally Boc_2O (2 equiv.) were added and the reaction stirred 10 hours. After this time the solution was extracted with diethyl ether (3x) and the combined organic fractions dried over anhydrous K_2CO_3. After evaporation of the solvents the solid residues were purified by silica gel flash chromatography (toluene isocratic).

General procedure B

In a sealed tube under argon at 0°C tetramethyldiaminomethane (4.5 equiv.) was carefully dissolved in trifluoroacetic acid [0.6 M] .After the addition of the desired olefin (1 equiv.), the tube was sealed and covered from visible light.

The sealed tube was heated to 75 °C for 15 hours before being quenched with a 1N aqueous solution of HCl (3 equiv.).After the resulting mixture was stirred 15 minutes, then an aqueous solution of 50 % NaOH was added dropwise until the pH became alkaline (pH 14). The solution was extracted with chloroform (4x) and the combined organic fractions were washed with brine and dried over anhydrous K_2CO_3.

After evaporation at reduced pressure of the volatiles, sulfamic acid was added directly to the crude (0.1equiv.). Distilled H_2O [1 M] and THF [1M] were, finally Boc_2O (2 equiv.) were added and the reaction stirred 10 hours. After this time the solution was extracted with diethyl

ether (3x) and the combined organic fractions dried over anhydrous K_2CO_3. After evaporation of the solvents the solid residues were purified by silica gel flash chromatography (toluene isocratic).

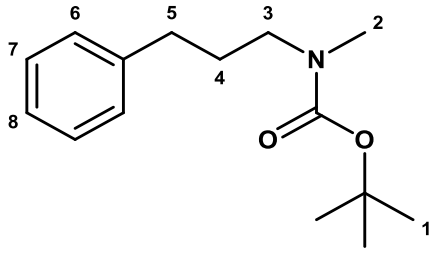


Figure 2 - *tert*-Butyl methyl(3-phenylpropyl)carbamate.

The *tert*-Butyl methyl(3-phenylpropyl)carbamate was isolated after silica gel flash chromatography purification in 60% yield as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ: 7.28–7.26 (m, 3H, *H*6, *8*), 7.19-7.15 (m, 2H, *H*7), 3.31-3.21 (m, 2H, *H*3), 2.84-2.81 (m, 3H, *H*2), 2.58 (t, *J* = 7.8 Hz, 2H, *H*5), 1.82 (q, *J* = 7.8Hz, 2H, *H*4), 1.42 (br s, 9H, *H*1).

^{13}CNMR (151 MHz, $CDCl_3$) δ: 155.97, 128.52, 128.43, 48.76, 48.38, 47.04, 34.27, 33.22, 29.68, 28.60.

HRMS: calculated for $C_{15}H_{23}NO_2[M+H]^+$: 250.1802 found: 250.3425

IR (neat, cm^{-1}): 3026, 2974, 2930, 2682, 1690, 1393.

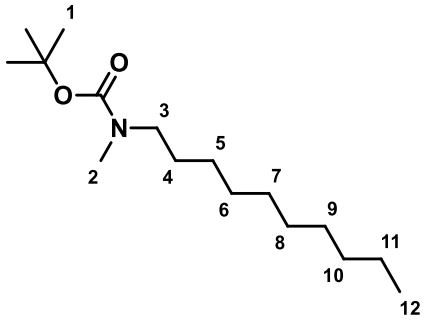


Figure 3-*tert*-Butyl decyl(methyl)carbamate.

The *Tert*-Butyl decyl(methyl)carbamate was purified by Silica gel flash chromatography with an isolated yield of 63% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ =3.17 (m, 2H, *H*3), 2.82 (s, 3H, *H*2),1.43 (q, *J*=6.6Hz, 2H, *H*4), 1.44 (br s, 9H, *H*1), 1.25 (m, 14H, *H*5-11) , 0.87 (t, *J*=6.6 Hz, 3H, *H*12)

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.11, 53.05, 52.48, 36.57, 34.64, 32.33, 31.28, 30.99, 28.62, 28.45, 26.11, 23.14, 22.64, 14.11, 14.09.

HMRS: calculated for: $C_{16}H_{33}NO_2[M+Na]^+$ m/z: 294.2404 found : 294.2407

IR (neat, cm^{-1}): 2923, 2854, 1695, 1392

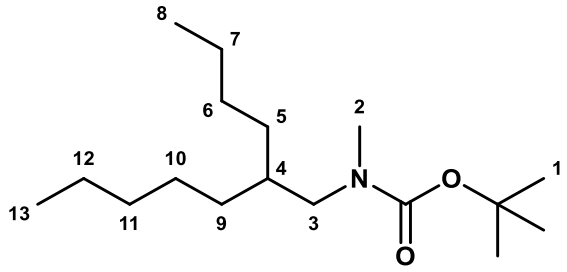


Figure 4-*tert*-Butyl (2-butylheptyl)(methyl)carbamate.

The *Tert*-Butyl (2-butylheptyl)(methyl)carbamate was isolated by silica gel flash chromatography with an isolated yield of 52% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.10 (m, 2H, H3), 2.81 (m, 3H, *H*2), 1.62 (m, 1H, *H*4), 1.45 (br s, 9H, *H*1), 1.33-1.18 (m, 14H, *H*5-7 and *H*9-12) 0.91- 0.86 (m, 6H, *H*8 and *H*13)

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.28, 53.21, 52.64, 36.74, 34.80, 32.49, 31.44, 31.15, 28.78, 28.61, 26.27, 23.30, 22.80, 14.27, 14.25.

HMRS: calculated for $C_{17}H_{35}NO_2Na^+$ m/z : 308.2560 found $[M+Na]^+$:308.2553

IR (neat,cm^{-1}):2921, 2852, 1698, 1420

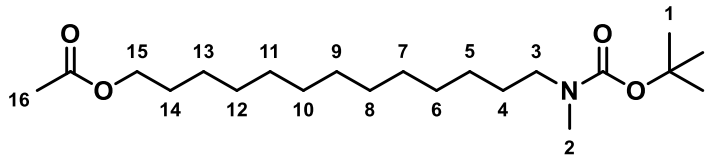


Figure 5-13-((*tert*-butoxycarbonyl)(methyl)amino)tridecyl acetate.

The 13-((*tert*-Butoxycarbonyl)(methyl)amino)tridecyl acetate was isolated by Silica gel flash chromatography with an isolated yield of 45% as an yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 4.01(t, *J*=6.6 Hz, 2H, *H*3), 3.12 (m, 2H, *H*15), 2.75 (br s, 3H, *H*2), 1.99 (s ,3H, *H*16), 1.57-1.55 (m, H2, *H*4), 1.44-1.42 (m, 2H, *H*14), 1.39(br s, 9H, *H*1), 1.19(m, 16H, *H*5-*H*13).

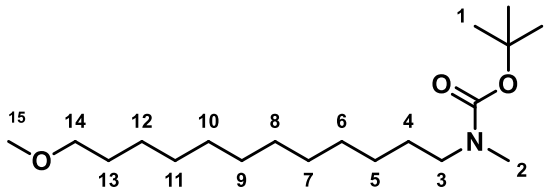


Figure 6-*tert*-Butyl(13-methoxytridecyl)(methyl)carbamate.

The *Tert*-Butyl(13-methoxytridecyl) (methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 54% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.38(t, *J*=6.6 Hz, 2H, *H*3), 3.20(m, 2H, *H*14), 2.85(br s, 3H, *H*15), 1.59-1.56(m, H2, *H*4), 1.5-1.48(m, 2H, *H*13), 1.45(br s, 9H, *H*1), 1.32-1.25(m, 16H, *H*5/*H12*)

^{13}CNMR (151 MHz, $CDCl_3$) δ = 155.88, 79.04, 72.99, 63.72, 58.57, 48.85, 48.49, 34.00, 30.32, 29.73, 29.68, 29.63, 29.59, 29.52, 29.39, 28.49, 27.88, 27.60, 26.72, 26.16.

HMRS: calculated for $C_{19}H_{39}NO_3$ $[M+Na]^+$ m/z : 352.6131 found $[M+Na]^+$:352.2817

IR (neat, cm^{-1}): 2924, 2854, 2261, 1174, 1692, 1478, 1414

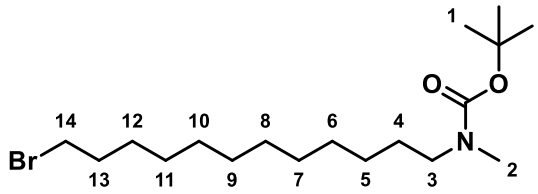


Figure 7-tert-Butyl (13-bromotridecyl)(methyl)carbamate.

The *tert*-Butyl (13-bromotridecyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 50% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.42-3.40(t, *J*=6.6 Hz, 2H, *H*3), 3.18(m, 2H, *H*14), 2.83(br s, 3H, *H*2), 1.88-1.83(q, *J*=7.2 Hz, 2H, *H*13), 1.50-1.47(m, 2H, *H*4), 1.45(b s, 9H, *H*1), 1.42-1.40(m, 2H, *12*), 1.32-1.22(m, 14H, *H*5/*H*12).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 165.99, 79.17, 48.97, 48.62, 34.28, 34.14, 32.98, 29.75, 29.69, 29.67, 29.58, 29.52, 28.91, 28.63, 28.32, 28.01, 26.84, 1.18.

HMRS: calculated for $C_{13}H_{23}NO_2$ $[M+Na]^+$ m/z : 400.1822 found $[M+Na]^+$:400.1821

IR (neat, cm^{-1}): 2973, 2924, 2853, 1697, 1458, 1393

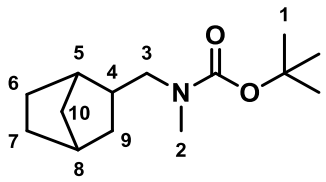


Figure 8-*tert*-Butyl (bicyclo[*2.2.1*]heptan-2-ylmethyl)(methyl)carbamate.

The *tert*-Butyl (bicyclo[2.2.1]heptan-2-ylmethyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 80% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.18-3.16(m, 1H), 2.84(br s, 3H, *H*2), 2.80-2.79(m, 1H), 2.21(m, 1H), 2.02(m, 1H), 1.73-1.71(m, 1H), 1.59(m, 1H), 1.60-1.59(m, 1H), 1.52-1.48(m, 2H), 1.43(br s, 9H, *H*1), 1.35-1.28(m, 5H), 1.13-1.10(m, 3H).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.18, 63.86, 53.32, 53.01, 40.75, 38.91, 38.55, 36.63, 35.37, 35.13, 34.83, 34.58, 34.10, 30.45, 29.86, 29.78, 29.19, 28.62, 27.87, 22.85, 14.29.

IR (neat, cm^{-1}): 2952, 2929, 2870, 1696, 1480, 1455

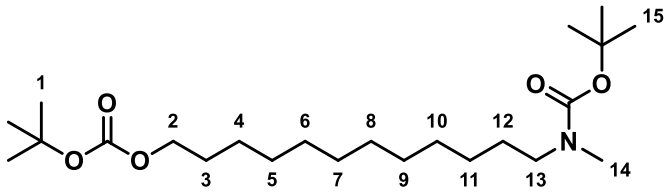


Figure 9-*tert*-butyl (13-((*tert*-butoxycarbonyl)oxy)tridecyl)(methyl)carbamate.

The *Tert*-Butyl (13-((*tert*-butoxycarbonyl) oxy)tridecyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 30% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 4.05-4.03(t, *J*=6.6 Hz, 2H, *H*13), 3.17(m, 2H, *H*2), 2.82(br s, 3H, *H*14), 1.66-1.60(q, *J*=7.2 Hz, 2H, *H*12), 1.48(br s, 9H, *H*1), 1.44(br s, 9H, *H*15), 1.35-1.33(m, 2H, *H*3), 1.27-1.25(m, 14H, *H*4/*H*11).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 153.85, 129.17, 128.36, 81.92, 79.17, 67.37, 48.97, 48.62, 34.13, 31.38, 29.75, 29.69, 29.68, 29.63, 29.52, 29.40, 28.84, 28.62, 28.01, 27.94, 26.83, 25.91, 21.61.

HMRS: calculated for $C_{23}H_{45}NO_5$ $[M+Na]^+$ m/z : 438.3905 found $[M+Na]^+$:438.3195

IR (neat, cm^{-1}): 2976, 2926, 2855, 1740, 1696, 1459

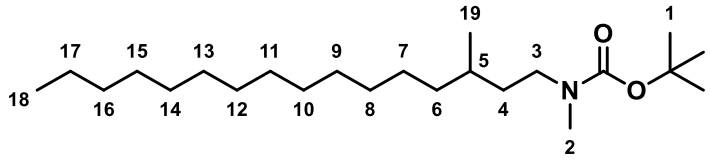


Figure 10-*tert*-Butyl methyl(3-methylhexadecyl)carbamate.

The *tert*-Butyl methyl(3-methylhexadecyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 25% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.2(m, 2H, *H*3), 3.83(br s, 3H, *H*2), 1.5-1.49(m, 1H, *H*5), 1.45(br s, 9H, *H*1), 1.29-1.25(m, 26H, *H*4 and H6-17), 0.89-0.86(m, 6H, *H18-19*),

^{13}CNMR (151 MHz, $CDCl_3$) δ = 128.65, 127.35, 79.19, 49.40, 47.16, 46.87, 37.13, 34.94, 34.63, 34.05, 32.08, 30.85, 30.49, 30.09, 29.84, 29.81, 29.52, 28.63, 28.59, 27.15, 22.85, 19.79, 14.29.

HMRS: calculated for $C_{23}H_{47}NO_2$ $[M+Na]^+$ m/z : 392.4107 found $[M+Na]^+$:392.3514

IR (neat, cm^{-1}): 2953, 2923, 2855, 1696, 1457, 1376

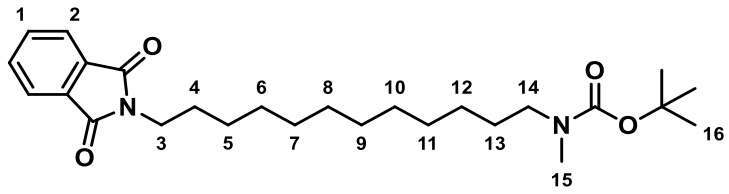


Figure 11-*tert*-Butyl (12-(1,3-dioxoisoindolin-2-yl)dodecyl)(methyl)carbamate.

The *tert*-Butyl (12-(1,3-dioxoisoindolin-2-yl)dodecyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 18% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.83-7.81(m, 2H, *H*2), 7.70-7.68(m, 2H, *H*1), 3.67-3.65(t, *J*=6.6 Hz, 2H, *H*3), 3.15(m, 2H, *H*14), 2.80(br s, 3H, *H15*), 1.65-1.62(m, 2H, *H*4), 1.46(br s, 9H, *H*16), 1.29(m, 4H, *H5*/13), 1.23(m, 14H, *H6-11*).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 168.58, 155.99, 133.95, 132.25, 123.25, 79.13, 63.13, 48.93, 48.57, 47.11, 40.73, 38.17, 34.49, 34.09, 32.91, 31.07, 30.16, 29.81, 29.69, 29.65, 29.58, 29.47, 29.40, 29.30, 28.72, 28.58, 28.54, 28.44, 27.96, 27.69, 26.97, 26.79, 25.85.

HMRS: calculated for $C_{19}H_{39}NO_3$ $[M+Na]^+$ m/z : 467.2988 found $[M+]^+$:467.2891

IR (neat, cm^{-1}): 2974, 2926, 2854, 1773, 1714, 1694, 1396

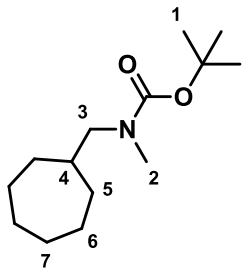


Figure 12-***tert*-Butyl (cycloheptylmethyl)(methyl)carbamate.**

The *Tert*-Butyl (cycloheptylmethyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 80% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.05-3.00(m, 2H, *H*3), 2.82(br s, 3H, *H*2), 1.76-1.75 (m, 1H, *H*4), 1.63-1.60(m, 6H, *H*5-6), 1.5-1.48(m, 2H, *H*6), 1.45(br s, 9H, *H*1), 1.40-1.38(m, 2H, *H*7), 1.13-1.12(m, H2, *H*7)

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.30, 55.01, 54.72, 37.91, 34.75, 34.44, 31.66, 28.87, 28.62, 28.55, 26.41, 26.24, 1.18.

HMRS: calculated for $C_{14}H_{27}NO_2$ $[M+Na]^+$ m/z : 264.5092 found $[M+Na]^+$:264.1935

IR (neat, cm^{-1}): 2974, 2922, 2854, 1695, 1480, 1395

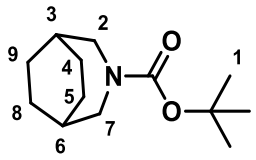


Figure 13- *tert*-butyl 4-azabicyclo[5.2.2]undecane-4-carboxylate.

The *Tert*-Butyl 4-azabicyclo[5.2.2]undecane-4-carboxylate was isolated by Silica gel flash chromatography with an isolated yield of 8% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 4.12-4.10(d,J=13.2 Hz, 1H, *H*2eq), 4.00-3.98(d, J=13.2 Hz, 1H, *H*9eq), 3.04-3.01 (d, J=12.6 Hz, 1H, *H*9ax), 2.95-2.93(d, J=12.6Hz, 1H, *H*2ax), 1.85-1.79(m, 4H),1.75-1.71(m, 2H), 1.63-1.61(m, 4H), 1.48(m, 2H), 1.45(br s, 9H)

^{13}CNMR (151 MHz, $CDCl_3$) δ = 162.48, 155.21, 153.31, 131.11, 128.84, 128.81, 128.06, 127.66, 127.20, 126.80, 126.03, 123.84, 82.65, 76.95, 69.99, 65.41, 64.56, 64.48, 54.56, 54.15, 49.72, 48.54, 35.18, 34.80, 34.63, 33.20, 33.11, 33.00, 32.84, 31.68, 31.32, 31.17, 29.84, 29.36, 28.87, 28.77, 28.72, 28.59, 28.27, 28.20, 27.85, 26.44, 25.77, 24.88, 22.81, 22.10, 20.68, 20.63, 20.51, 14.27, 10.32.

IR (neat, cm^{-1}): 2923, 2854, 1695, 1392

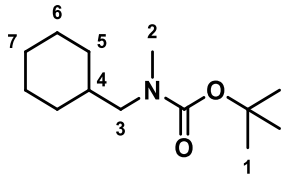


Figure 14-*tert*-butyl (cyclohexylmethyl)(methyl)carbamate.

The *tert*-butyl (cyclohexylmethyl)(methyl)carbamate.was isolated by Silica gel flash chromatography with an isolated yield of 18% as yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.04-3.02(m, 2H, *H*3), 2.84-2.83(br s, 3H, *H*1),1.73-1.71(m, 2H, *H*5), 1.65-1.63(m, 2H, *H*5), 1.57(m, 1H, *H*4), 1.45(br s, 9H, *H*1), 1.22-1.16(m, 4H, *H*6), 0.90-0.88(m, H2, *H*7) .

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.26, 64.58, 55.42, 54.98, 36.96, 36.57, 35.28, 34.88, 30.95, 30.83, 28.62, 27.87, 26.66, 26.08.

IR (neat, cm^{-1}): 2974, 2925, 2853, 1746, 1696, 1451

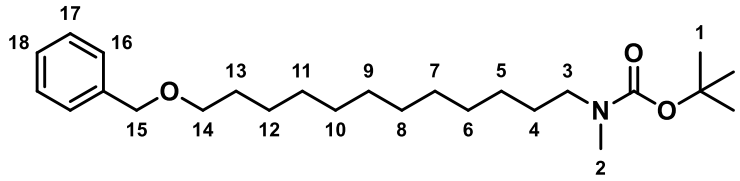


Figure 15-*tert*-Butyl (12-(benzyloxy)dodecyl)(methyl)carbamate.

The *tert*-Butyl (12-(benzyloxy)dodecyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 40 % as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.34-7.33(m, 3H, *H*16/18), 7.28-7.27(m, 1H, *H*17), 4.50(s, 2H, *H*15), 3.46(t, *J*=6.6 Hz, 2H, *H*3), 3.17(m, 2H, *H*14), 2.83(br s, 3H, *H*2), 1.63-1.58(q, J=6.6 Hz 2H, *H*13), 1.48(m, 2H, *H*4), 1.45(br s, 9H, *H*1), 1.35(m, 2H, *H*12), 1.25(m, 14H, *H*5-11).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 138.83, 128.48, 127.76, 127.60, 79.17, 73.00, 70.67, 48.98, 34.13, 30.45, 29.93, 29.86, 29.77, 29.74, 29.64, 29.52, 28.63, 28.01, 26.84, 26.34.

HMRS: calculated for: : $C_{25}H_{43}NO_3$ $[M+Na]^+$ m/z: 428.629 found : 428.131

IR (cm^{-1}): 3418, 2925, 2854, 1696, 1677, 1397, 1366

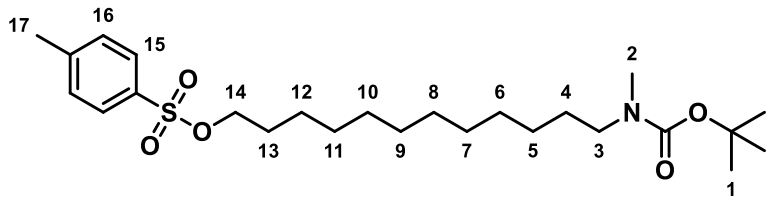


Figure 16-12-((*tert*-Butoxycarbonyl)(methyl)amino)dodecyl 4-methylbenzenesulfonate.

12-((*tert*-Butoxycarbonyl)(methyl)amino)dodecyl 4-methylbenzenesulfonate was isolated by Silica gel flash chromatography with an isolated yield of 30% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.79-7.78(d, J=9 Hz , 2H, *H*15), 7.35-7.33(d, J=9 Hz, 2H, *H*16), 4.02-4.00(t, *J*=6.6 Hz, 2H, *H*3), 3.17(m, 2H, *H*14), 2.83(br s, 3H, *H*2), 2.45(s, 3H, *H*17),

1.65-1.60(q, J=6.6 Hz, 2H, *H*13), 1.48(m, 2H, *H*4), 1.45(br s, 9H, *H*1), 1.30-1.16(m, 16H, *H*5/12).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 144.75, 133.31, 129.93, 128.03, 79.17, 70.86, 48.97, 48.61, 34.13, 29.74, 29.67, 29.62, 29.53, 29.08, 28.95, 28.62, 28.34, 28.02, 26.84, 25.47, 21.80.

HMRS: calculated for: : $C_{25}H_{43}NO_5S$ $[M+Na]^+$ m/z: 492.5912 found : 492.2749

IR (cm^{-1}): 2924, 2854, 1690, 1458, 1362, 1174

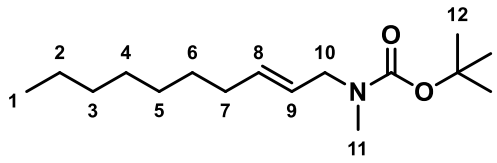


Figure 17-*tert*-Butyl (E)-dec-2-en-1-yl(methyl)carbamate.

tert-Butyl (E)-dec-2-en-1-yl(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 45% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 5.51(m, 1H, *H*9), 5.36(m, 1H, *H*8), 3.72(m, 2H, *H*10), 2.78(s, 3H, *H*11), 2.01-1.99(m, 2H, *H*7), 1.44(br s, 9H, *H*12), 1.36-1.34(m, 2H, *H*6), 1.26(m, 8H, *H*2-5), 0.88-0.86(t, 3H, *H*1).

^{13}CNMR (151 MHz, $CDCl_3$) δ= 155.86, 134.07, 133.58, 125.10, 79.35, 51.03, 50.26, 33.49, 32.35, 31.97, 31.09, 29.39, 29.28, 29.22, 28.59, 28.44, 22.79, 14.24.

IR (cm^{-1}): 2958, 2926, 2855, 1699, 1545, 1392, 1365

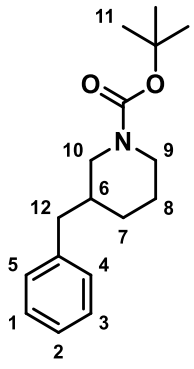


Figure 18-*tert*-Butyl 3-phenylpiperidine-1-carboxylate.

tert-Butyl 3-phenylpiperidine-1-carboxylate was isolated by Silica gel flash chromatography with an isolated yield of 17% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.34-7.32(m, 2H, *H*1/*H*3), 7.26-7.24(m, 1H, *H*2), 7.21-7.20(d, *J*=7.2 Hz, 2H, *H*4-5), 4.13-3.80(m, 2H), 2.85(m, 1H), 2.64-2.51(m, 3H), 1.80(m, 2H), 1.66(m, 1H), 1.48(br s, 9H, *H*11), 1.16(m, 1H).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 155.04, 140.05, 129.17, 128.39, 126.10, 79.36, 64.58, 63.85, 37.85, 30.76, 29.85, 28.56, 27.86.

HMRS: calculated for: $C_{17}H_{25}NO_3$ $[M+Na]^+$ m/z: 298.1885 found : 298.1780

IR (neat, cm^{-1}): 2975, 2927, 2850, 1687, 1418, 1364

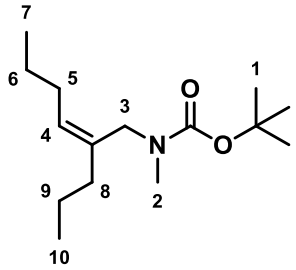


Figure 19-*tert*-Butyl (Z)-methyl(2-propylhex-2-en-1-yl)carbamate.

Tert-Butyl (Z)-methyl(2-propylhex-2-en-1-yl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 30% as yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 5.21(m, 1H, *H*4), 3.77-3.73(m, 2H, *H*3), 2.74-2.70(m, 2H, *H*8), 2.78(s, 3H, *H*2), 2.05-2.02(q, *J*= 6.6 Hz, 2H, *H*5), 1.93-1.90(t, *J*=7.2 Hz, 3H, *H*7), 1.45(br s, 9H, *H*1), 1.40-1.35(m, 4H, *H*6/9), 0.91-0.88(m, 6H, *H*9-10).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.02, 135.03, 128.01, 127.76, 79.37, 54.52, 53.87, 33.14, 30.39, 30.10, 29.76, 28.57, 23.13, 21.68, 14.33, 13.99.

HMRS: calculated for: $C_{15}H_{29}NO_2$ $[M+Na]^+$ m/z: 278.2198 found : 278.2021

IR (neat, cm^{-1}): 2959, 2929, 2871, 1694, 1392, 1366, 1243

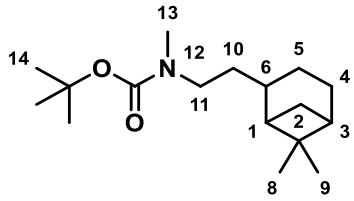


Figure 20-*tert*-Butyl (2-(6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethyl)(methyl)carbamate.

Tert-Butyl (2-(6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 5% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.91-3.2(m, 2H, *H*11), 2.73(s, 3H, *H*13), 2.53-2.31(m, 1H, *H*6), 1.93-1.85(m, 5H), 1.59-1.57(m, 2H, *H*10), 1.57(br s, 9H, *H*14), 1.18(s, 3H, *H*8), 1.00(s, 3H, *H*9).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 155.86, 129.17, 128.36, 125.43, 47.76, 46.34, 41.52, 41.07, 38.82, 38.60, 37.12, 35.66, 34.08, 33.75, 32.56, 29.86, 28.64, 28.32, 27.56, 26.93, 26.57, 24.78, 24.65, 23.62, 23.43, 22.47, 21.62, 20.26, 19.99, 1.18.

HMRS: calculated for: : $C_{17}H_{31}NO_2$ $[M+Na]^+$ m/z:304.2855 found : 304.2250

IR (neat, cm^{-1}): 2929, 2867, 2815, 2763, 1696, 1459, 1394

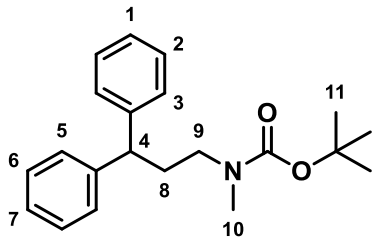


Figure 21-*tert*-Butyl (3,3-diphenylpropyl)(methyl)carbamate.

Tert-Butyl (3,3-diphenylpropyl)(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 20% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.31-7.25(m, 9H, *H*2/3 and *H*5/6), 3.897(m, 1H, *H*4), 3.18(m, 2H, *H*9), 2.84(br s, 3H, *H*10), 2.29(m, 2H, *H*8), 1.46-1.41(br s, 9H, *H*11).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 155.73, 144.36, 143.99, 141.88, 139.12, 129.80, 128.23, 128.20, 127.73, 127.66, 127.59, 126.34, 79.33, 60.70, 49.20, 48.69, 47.87, 34.60, 34.19, 34.16, 33.60, 33.32, 30.45, 30.35, 29.75, 28.47, 25.51, 22.56, 22.39, 14.14.

HMRS: calculated for: : $C_{21}H_{27}NO_2$ $[M+Na]^+$ m/z: 348.2542 found : 348.193

IR (neat, cm^{-1}): 2974, 2928, 2869, 1693, 1492, 1394, 1168

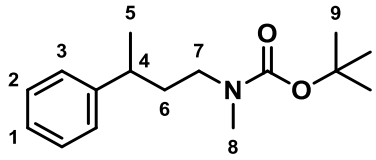


Figure 22-*tert*-Butyl methyl(3-phenylbutyl)carbamate.

tert-Butyl methyl(3-phenylbutyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 15% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.31-7.28(t, *J*=7.2 Hz, 2H, *H*2), 7.19-7.18(d, 3H, *H*1/3), 3.11-3.05(m, 2H, H7), 2.80-2.75(m, 3H, *H*8), 2.67(m, 1H, *H*4), 1.84-1.75(m, 2H, *H*6), 1.41(br s, 9H, *H*9), 1.27-1.26(d, *J*=6.6 Hz, 3H, *H*5).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 155.87, 128.59, 126.96, 126.21, 79.30, 47.62, 37.97, 37.59, 36.05, 35.81, 34.14, 29.85, 28.58, 22.73, 22.49.

HMRS: calculated for: : $C_{16}H_{25}NO_2$ $[M+Na]^+$ m/z: 286.1885 Found: 286.1765

IR (cm^{-1}): 2961, 2926, 2856, 1696, 1453, 1174

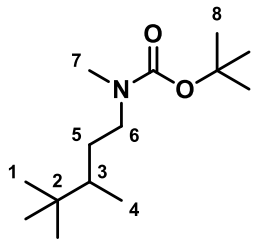


Figure 23-*tert*-Butyl methyl(3,4,4-trimethylpentyl)carbamate.

Tert-Butyl methyl(3,4,4-trimethylpentyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 40% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.21-3.13(m, 2H, *H*6), 2.83(s, 3H, *H*7), 1.75-1.73(m, 1H, *H*3), 1.45(s, 9H, *H*8), 1.06(m, 2H, *H*5), 0.86-0.84(br s, 12H, *H*1/4).

^{13}CNMR (151 MHz, $CDCl_3$) δ = 156.18, 79.23, 64.58, 48.46, 48.05, 40.26, 34.11, 33.12, 30.14, 29.60, 28.62, 28.48, 27.87, 27.36, 14.52.

HMRS: calculated for: : $C_{14}H_{29}NO_2$$[M+Na]^+$ m/z: 266.2198 found: 266.2089

IR (cm^{-1}):2963, 2869, 1696, 1477, 1423, 1393

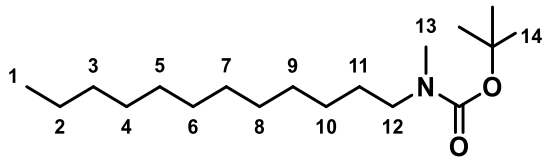


Figure 24-*tert*-Butyl dodecyl(methyl)carbamate.

tert-Butyl dodecyl(methyl)carbamate was isolated by Silica gel flash chromatography with an isolated yield of 77% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 3.17(m, 2H, *H*12), 2.83(s, 3H, *H*13), 1.49-1.47(m, 2H, *H*11), 1.45(br s, 9H, *H*14),1.29-125 (m, 18H, *H*2/10), 0.88-0.86(t, *J*=6.6 Hz 2H, *H*1)

^{13}CNMR (151 MHz, $CDCl_3$) δ = 151.92, 79.17, 63.86, 48.98, 34.12, 32.07, 29.82, 29.78, 29.76, 29.50, 28.63, 28.01, 26.83, 22.85, 14.29.

IR (neat, cm^{-1}): 2923, 2854, 1695, 1392

V.1.2-General procedure for hydroaminomethylation using Acetic Acid/Sulfuric Acid and Tetramethyldiaminomethane

In a sealed tube under argon at 0°C tetramethyldiaminomethane (1.5 equiv.) was carefully dissolved in Acetic acid [0.6 M] and H_2S0_4 (1 equiv.) .After the addition of the desired olefin (1 equiv.), the tube was sealed and covered from visible light.

The sealed tube was heated to 75°C for 15 hours before being quenched with a 1N aqueous solution of HCl (3 equiv.).After the resulting mixture was stirred 15 minutes, then an aqueous solution of 50 % NaOH was added dropwise until the pH became alkaline (pH 14). The solution was extracted with chloroform (4x) and the combined organic fractions were washed with brine and dried over potassium carbonate.

Literature reference[54]

V.1.3-General procedure for hydroaminomethylation using Acetic Acid and Eschenmoser's salt

In a sealed tube under Argon 1.5 equiv. of Eschenmoser's salt were weight in before the addition of Acetic Acid [0.2 M]. After stirring for 15 minutes the olefin was added and the solution heated to 75 ºC for 15 hours.

An aqueous solution of 50 % NaOH was added dropwise until the pH became alkaline (pH 14). The solution was extracted with chloroform (4x) and the combined organic fractions were washed with brine and dried over potassium carbonate.

V.1.4-General procedure for aminomethylation using ACN and Eschenmoser's salt

In a sealed tube under Argon 1.5 equiv. of Eschenmoser's salt was weight in before the addition of ACN [0.2 M]. After stirring for 15 minutes the olefin was added and the solution heated to 75 ºC for 15 hours.

An aqueous solution of 50 % NaOH was added dropwise until the pH became alkaline (pH 14). The solution was extracted with chloroform (4x) and the combined organic fractions were washed with brine and dried over potassium carbonate.

Literature reference[54]

V.1.5-General procedure of aminomethylation using of Acetyl Chloride and Tetramethyldi-aminomethane

A freshly distilled acetyl chloride (1equiv.) was added to a solution of Tetramethyldia-minomethane and [1M] DCM at 0 °C. After 15 minutes a solution of the olefin in DCM [2M] was then added to the mixture. The solution was rotary evaporated and extracted with EtOAc (3x). The combined organic phases were washed with brine. The yield was calculated by ^{1}HNMR using 1, 3, 5 trimetoxybenzene as an internal standard.

Procedure in accordance with literature (Millot, Piazza, Avolio, & Knochel, 2000)

V.1.6-General procedure of hydroaminomethylation using trioxane and Dimethylamine

V.2-Starting Material synthesis

V.2.1-Wittig olefination's general procedure

In a flamed dried flask under argon, 1.1 equiv. phosphonium salt with 0.25 M of dry THF. The solution was then stirred at -78 °C. The additions of 1.2 equiv. of *n*BuLi [1.6M] lead to a change of color. After 35 minutes the ketone / aldehyde / enone was added dropwise. The solution was left to warm until room. The reaction was followed by TLC using $KMn0_4$ as a revelator.

When complete, ammonium Chloride and a solution of NaOH (10%) were added to quench the reaction and to prevent the isomerization of the olefin product. The solution was extracted 3 times with diethyl ether and the combined organic phases were extracted with brine and dried using $MgSO_4$. The crude was purified using silica flash gel chromatography (heptane/ 0.5% diethyl ether).

V.2.3-Synthesis of a terminal bromo alkene by appel reaction

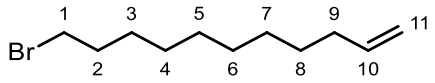


Figure 25-11-bromoundec-1-ene.

The 11-bromoundec-1-ene was isolated by Silica gel flash chromatography with an isolated yield of 80% as a yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 5.83-5.78(m, 1H, *H*10), 5.00-4.92(m, 2H, *H*11), 3.42-3.40(t, *J*=7.8 Hz, 2H, *H*1), 2.05-2.02(q, J=7.2 Hz, 2H, *H*9), 1.87-1.83 (qui,J=6.6 Hz, H2 , *H*2), 1.43-1.41(m, 2H, *H*3), 1.38-1.36(m, 2H, *H*8), 1.28 (m, 8H, *H*4-7)

Experimental procedure and spectral data in accordance to the literature[75] :

V.2.4 – Acetylation of undecene–1-ol

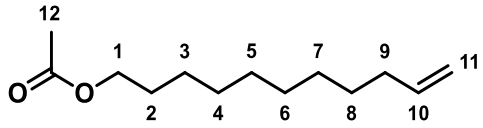


Figure 26-undec-10-en-1-yl acetate.

The Undec-10-en-1-yl acetate was isolated by Silica gel flash chromatography with an isolated yield of 45% as yellow oil.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 5.83-5.78 (m, 1H, *H*10), 5.00-4.91(m, 2H, *H*11), 2.042 (br s, 3H, *H*12), 1.62-1.59 (m, 2H, *H*9), 1.38-1.27 (m, H14, *H*3/8).

Experimental procedure and spectral data in accordance with the literature: [76]

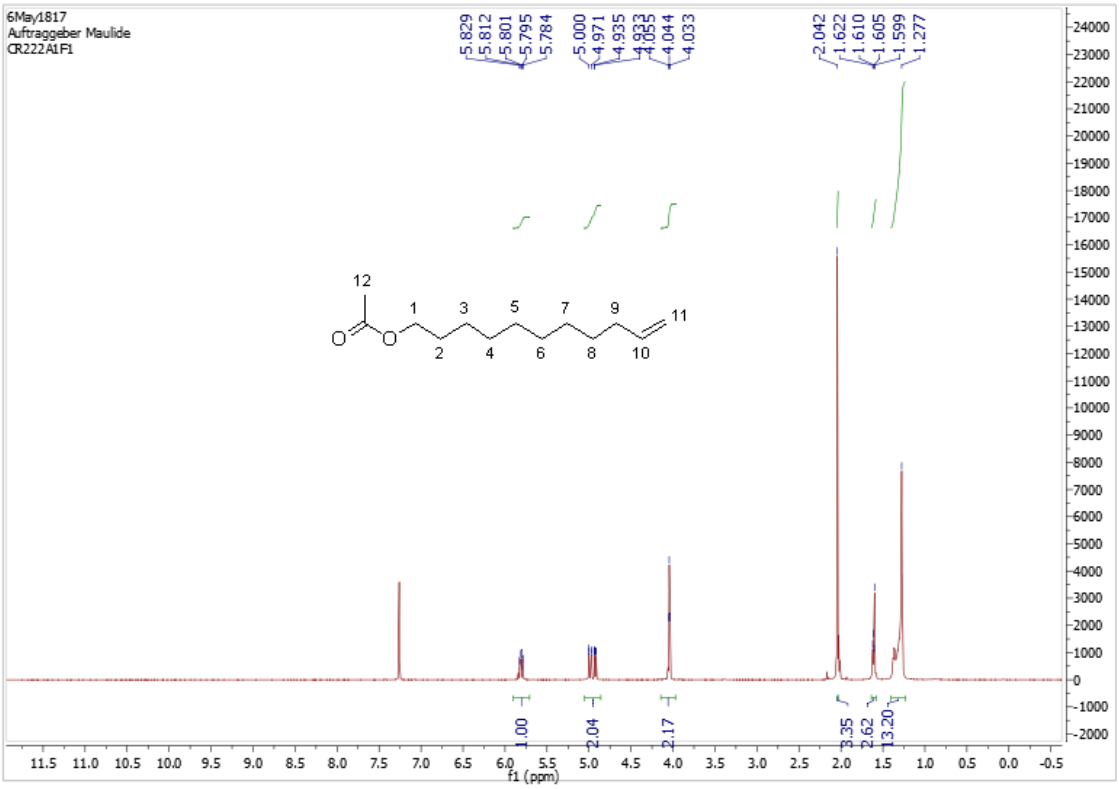


Figure 27-^{1}HNMR spectra of undec-10-en-1-yl acetate.

V.2.4-Substitution of bromine with potassium Pthalamine

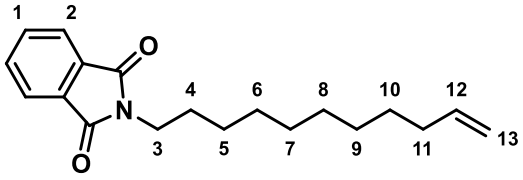


Figure 28-2-(undec-10-en-1-yl)isoindoline-1,3-dione.

The 2-(undec-10-en-1-yl)isoindoline-1,3-dione was isolated by Silica gel flash chromatography with an isolated yield of 60% as a yellow solid.

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.85-7.83(m, 2H, *H*2), 7.71-7.70(m, 2H, *H*1), 5.82-5.78(m, 1H, *H*12), 3.75(dd, *J*=17.4 Hz, 2H, *H*13), 3.68-3.66(t, *J*=7.2 Hz, 2H, *H*3), 1.67-1.65(m, 2H, *H*11), 1.35-1.31(m, 6H, *H*4/5/10), 1.26(m, 6H, *H*5/9).

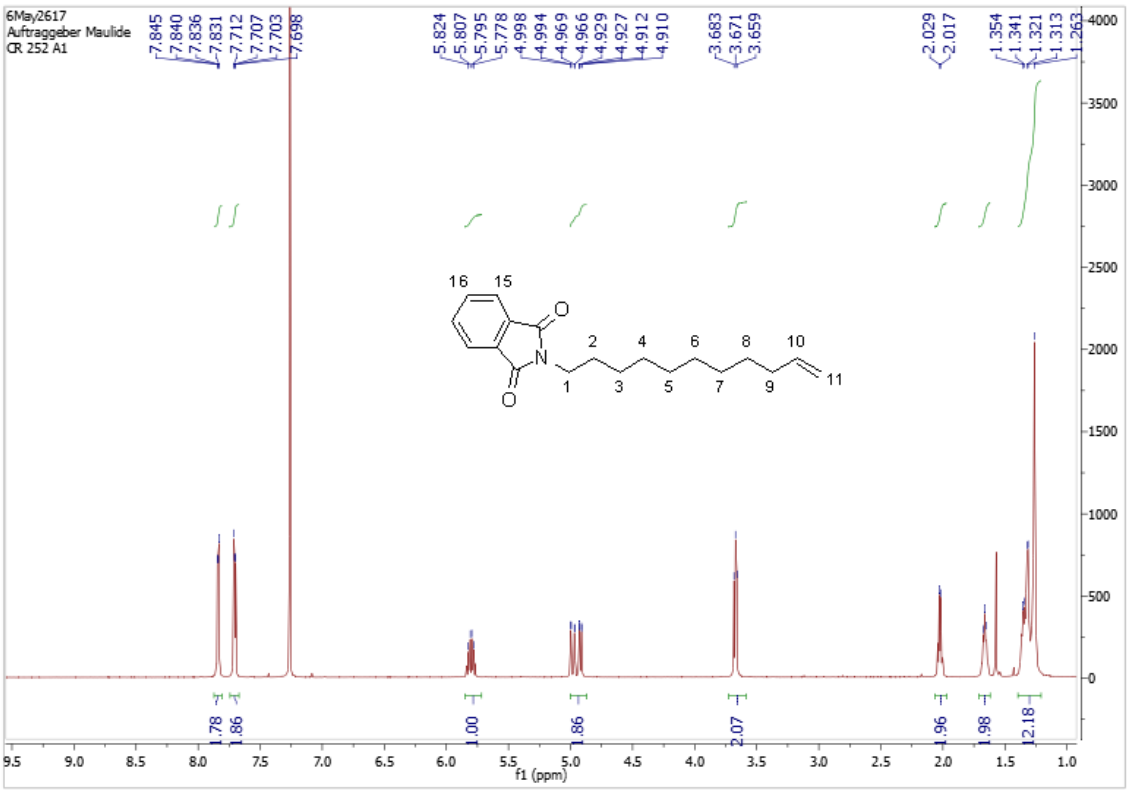


Figure 29-^{1}HNMR spectra of 2-(undec-10-en-1-yl)isoindoline-1,3-dione.

Experimental procedure and spectral data are in accordance with the literature[76].

V.2.5-Methylation of Undecene-1-ol

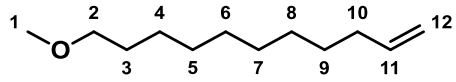


Figure 30 - 11-methoxyundec-1-ene

Undecene-1-ol was bought from TCI chemicals. Experimental procedure and spectral data in accordance with the literature[77]

^{1}HNMR (600 MHz, $CDCl_3$) δ = 5.85-5.78(m, 1H, *H*11), 5.01-4.94(m, 2H, *H*12), 3.37-3.32 (m, 2H, *H*2), 3.33-3.32(m, 2H, *H*1), 2.58(s, 3H, *H1*), 2.05-2.03(m, 2H, *H*3), 1.57-1.52(m, 2H, *H*9), 1.29(m, 14H, *H*4/8), 1.26(m, 10H, *H*5/9).

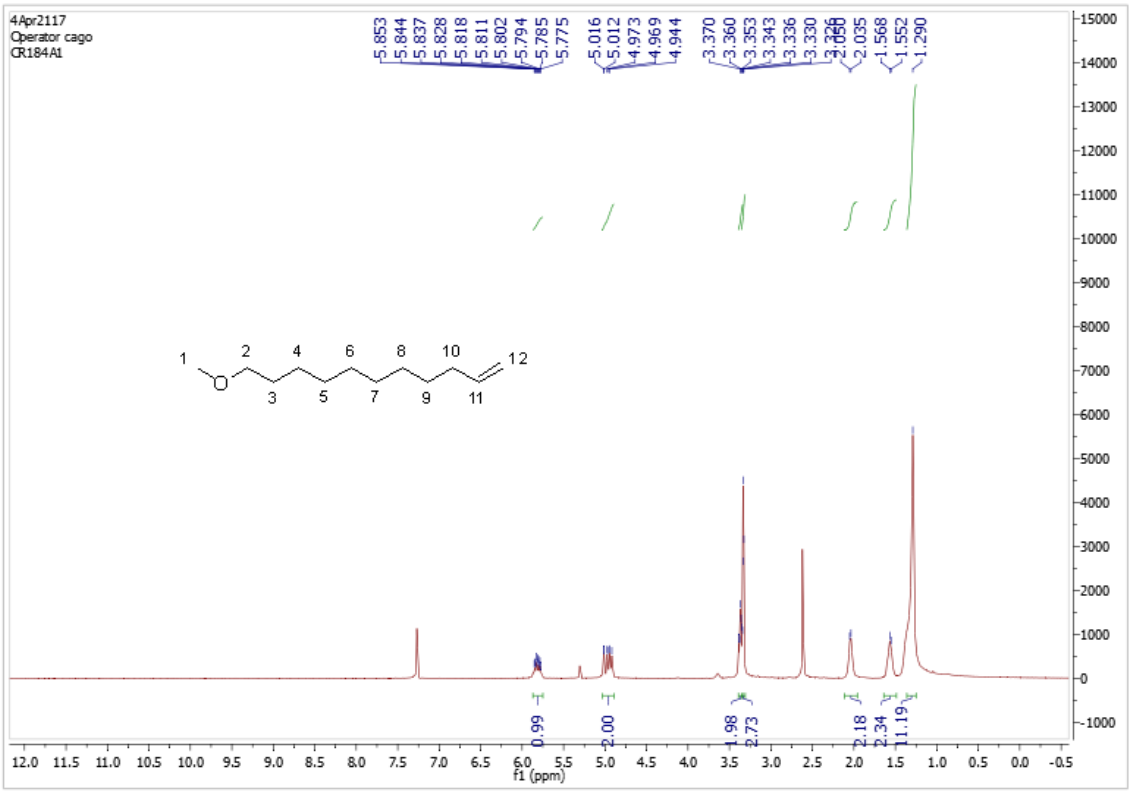


Figure 31-^{1}HNMR of 11-methoxyundec-1-ene.

V.2.6-Sylilation of Undecene-1-ol

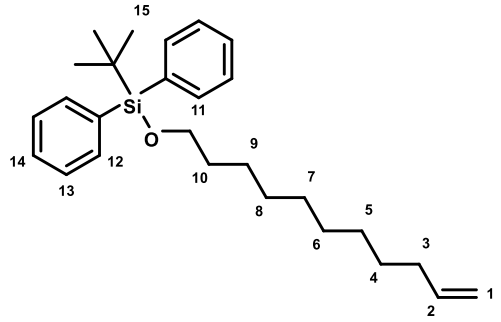


Figure 32 - *tert*-butyldiphenyl(undec-10-en-1-yloxy)silane

Spectral data and experimental procedure in accordance with Literature[78]

^{1}HNMR (600 MHz, $CDCl_3$) δ = 7.68-7.66(m, 4H, *H*14), 7.42-7.37(m, 6H, *H*13/15), 5.83-5.81 (m, 1H, *H*2), 5.02-4.92(m, 2H, *H*1), 3.67-3.64(t, *J*=7.8 Hz, 2H, *H11*), 2.05-2.03(q, *J*=6.4 Hz, 2H, *H*3), 1.56-1.53(m, 2H, *H10*), 1.37-1.34(m, 4H, *H*4/9), 1.26(m, 9H, *H*12), 1.06-1.05(m, 8H, *H*5/8).

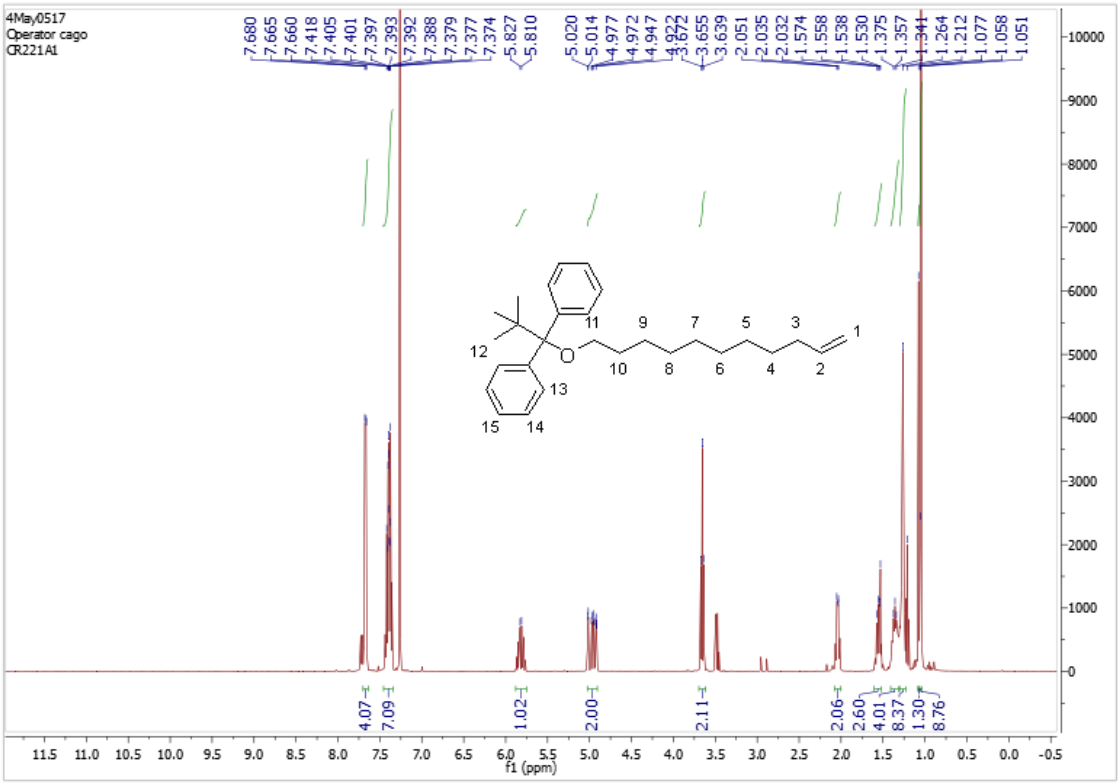


Figure 33- ^{1}HNMR of *tert*-butyldiphenyl(undec-10-en-1-yloxy)silane

V.3-Synthesis of tetraalkyldiaminomethane

V.3.1-Synthesis of tetrabenzyldiaminomethane

A solution of 1M ethanol and paraformaldehyde was heated with the heating gun to dissolve the para-formaldehyde. 1.9 equivalents of Dibenzylamine were added before the solution was heated to 50 ºC. After 17 hours the solution was concentrated and put on ice.The solid was then vacuum filtered and washed with water and cold ethanol.

Procedure in accordace with literature [79]

V.3.2-Procedure for the synthesis of tetraethyldiaminomethane

A sealed tube with 1 equivalent of paraformaldehyde and [10 M] H_20 was heated until the paraformaldehyde was dissolved. 1.9 equiv.of previously distilled diethylamine were added, followed by 5 equiv. of NaOH.

The solution was then heated to 75ºC and quenched with brine. The aqueous phase was extracted with (3x) diethylether and the combined organic phases dried with anhydrous K_2CO_3

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Appendix

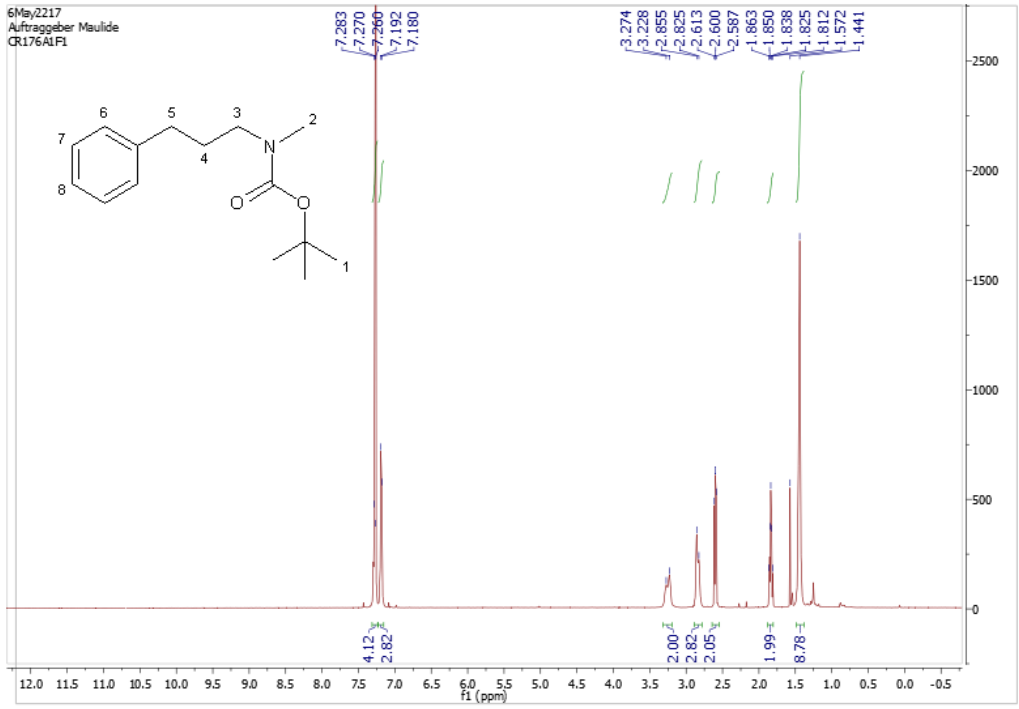

Figure 34 - ^{1}H NMR spectra of *tert*-Butyl methyl(3-phenylpropyl)carbamate (5.2).

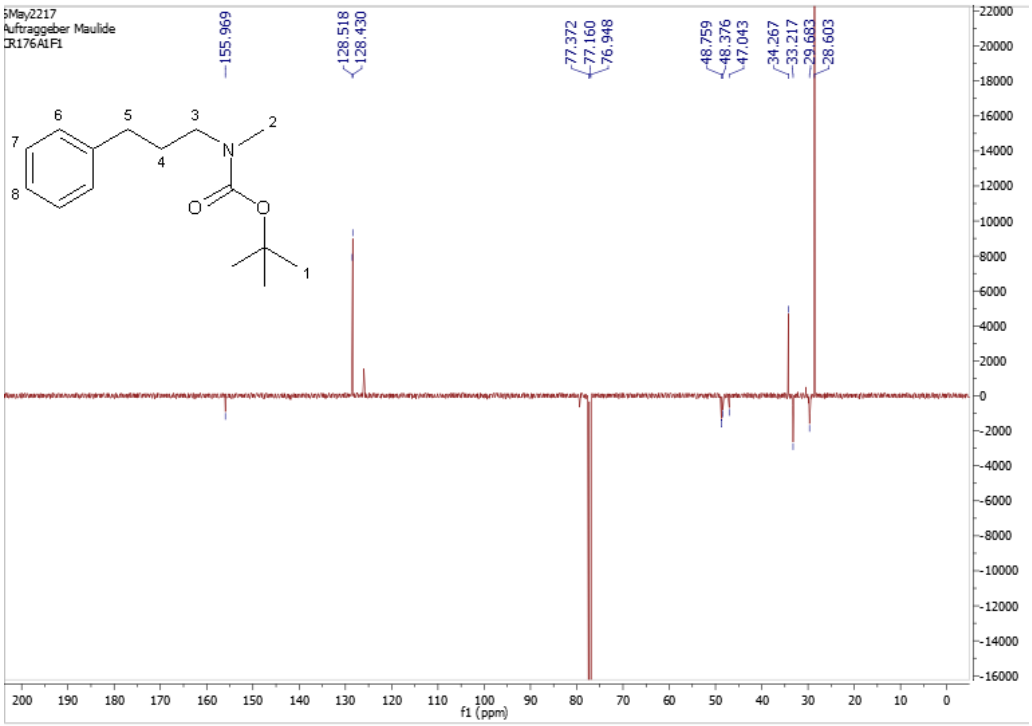

Figure 35 - ^{13}C NMR spectra of *tert*-Butyl methyl (3-phenylpropyl)carbamate (5.2).

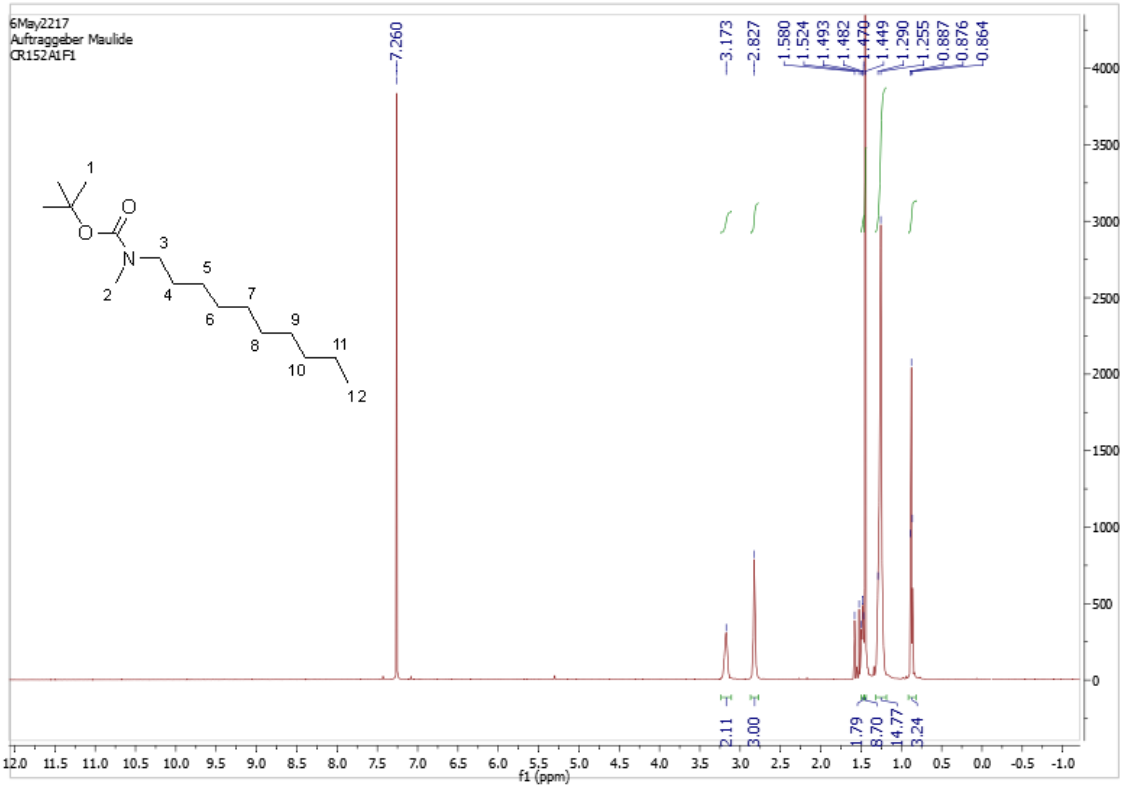

Figure 36 - ^{1}H NMR spectra of *tert*-Butyl decyl(methyl)carbamate (5.3).

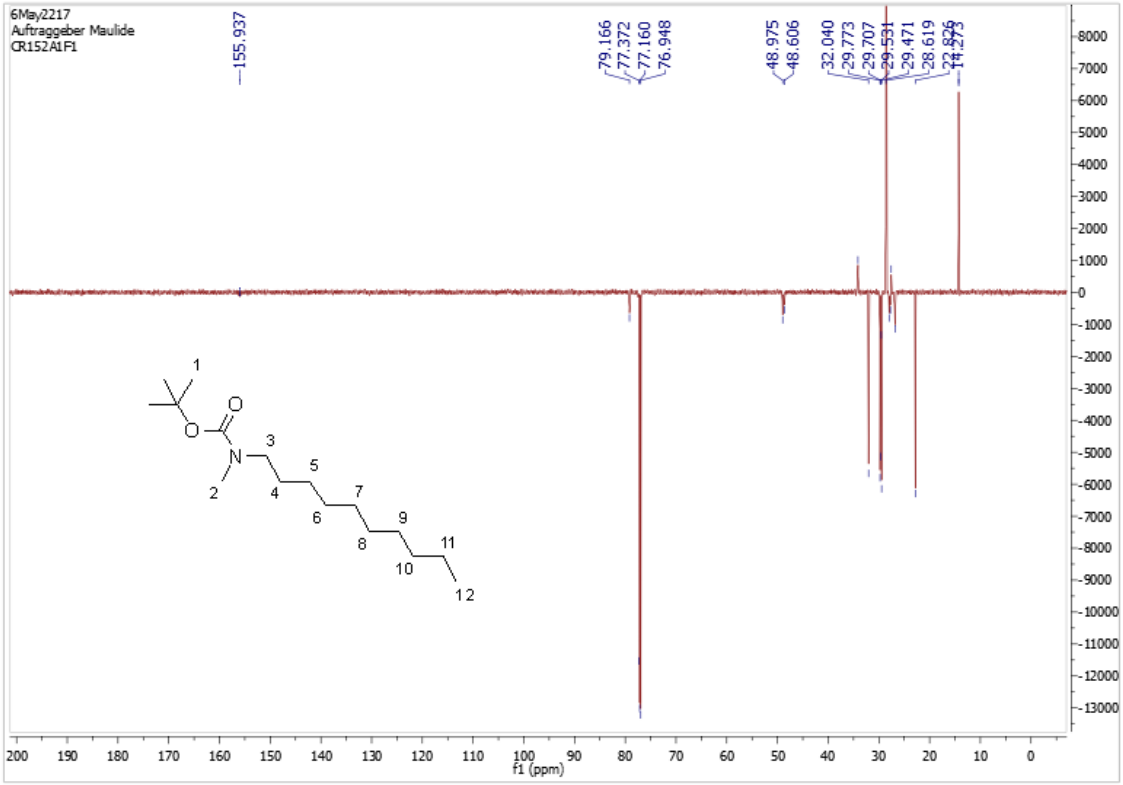

Figure 37 - ^{13}C NMR spectra of *tert*-Butyl decyl(methyl)carbamate (5.3).

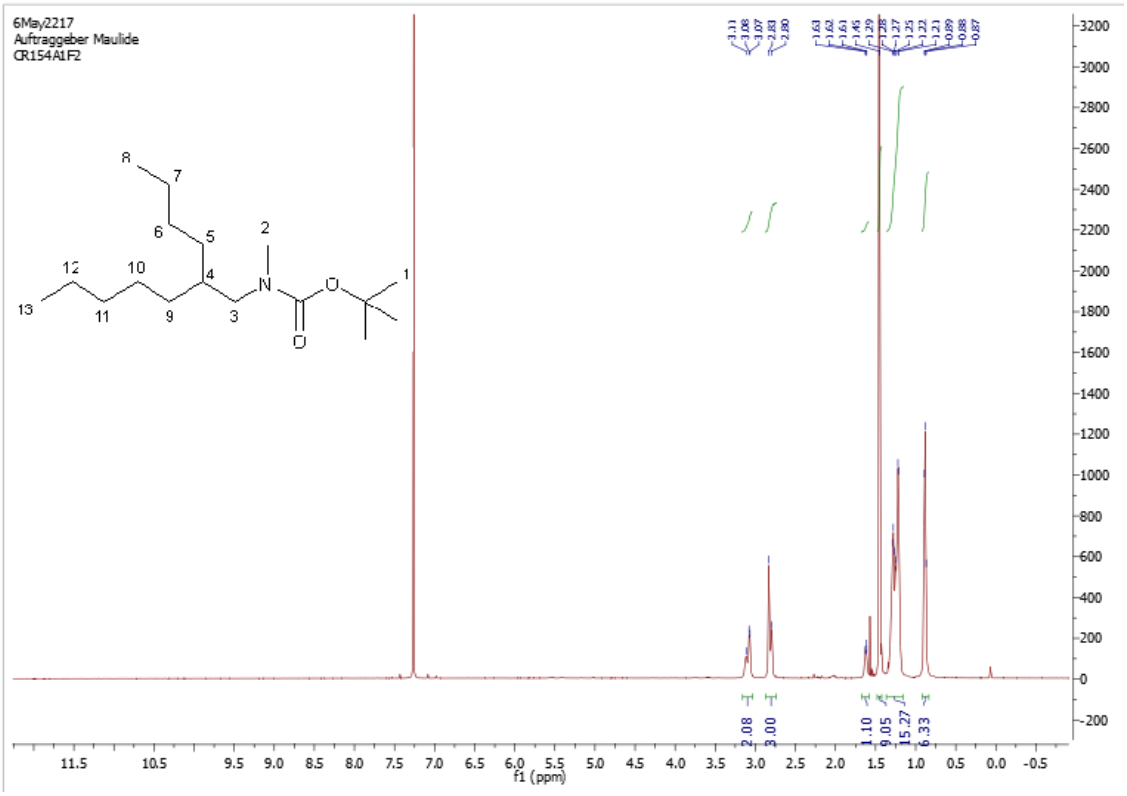

Figure 38 - ^{1}H NMR spectra of *tert*-Butyl (2-butylheptyl)(methyl)carbamate(5.6/ 5.7).

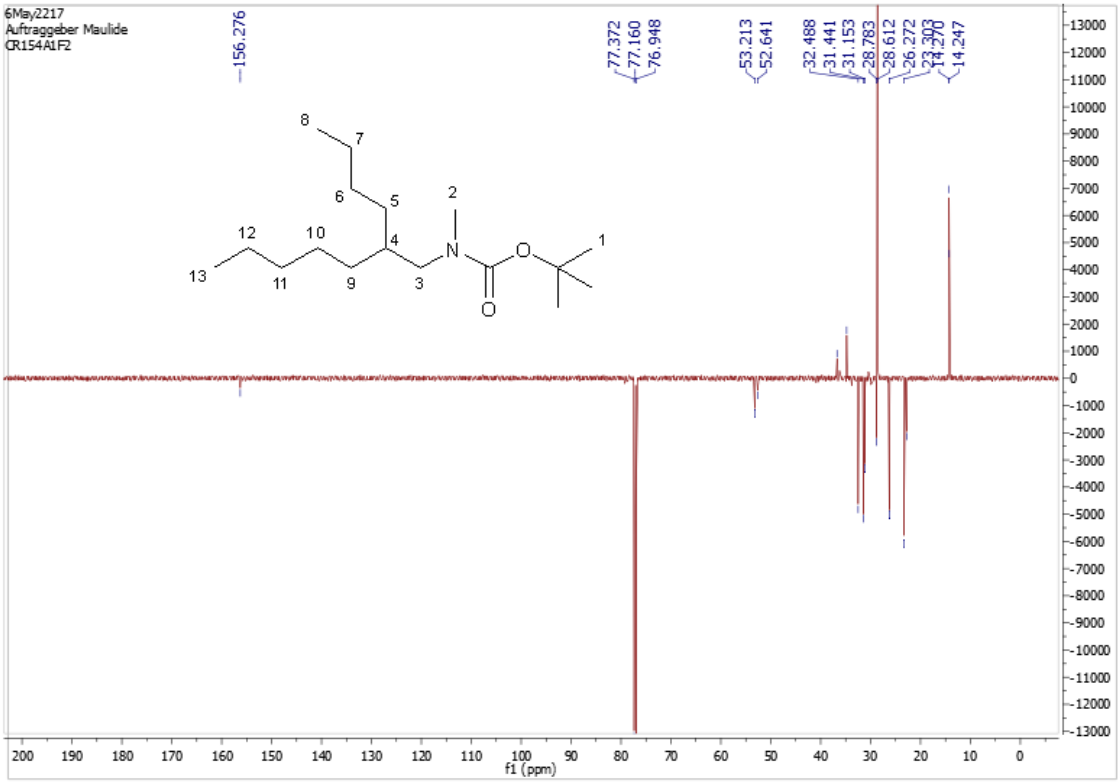

Figure 39 - ^{13}C NMR spectra of *tert*-Butyl (2-butylheptyl)(methyl)carbamate (5.6/ 5.7).

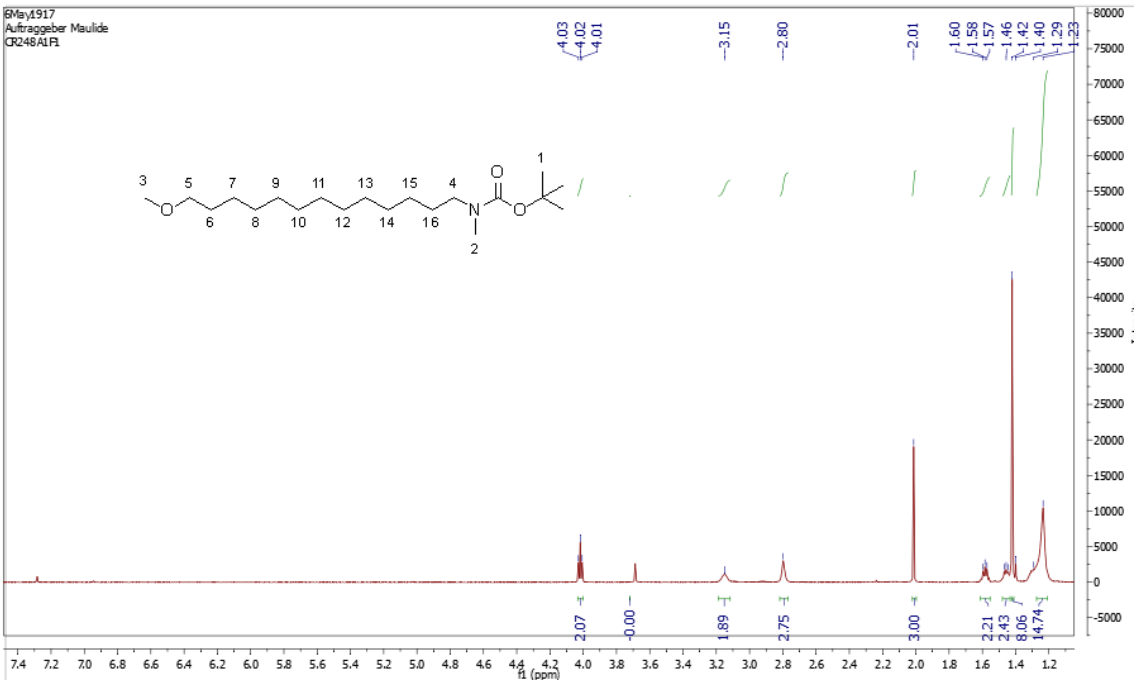

Figure 40 - ^{1}H NMR spectra of *tert*-Butyl (13-methoxytridecyl)(methyl)carbamate (5.14).

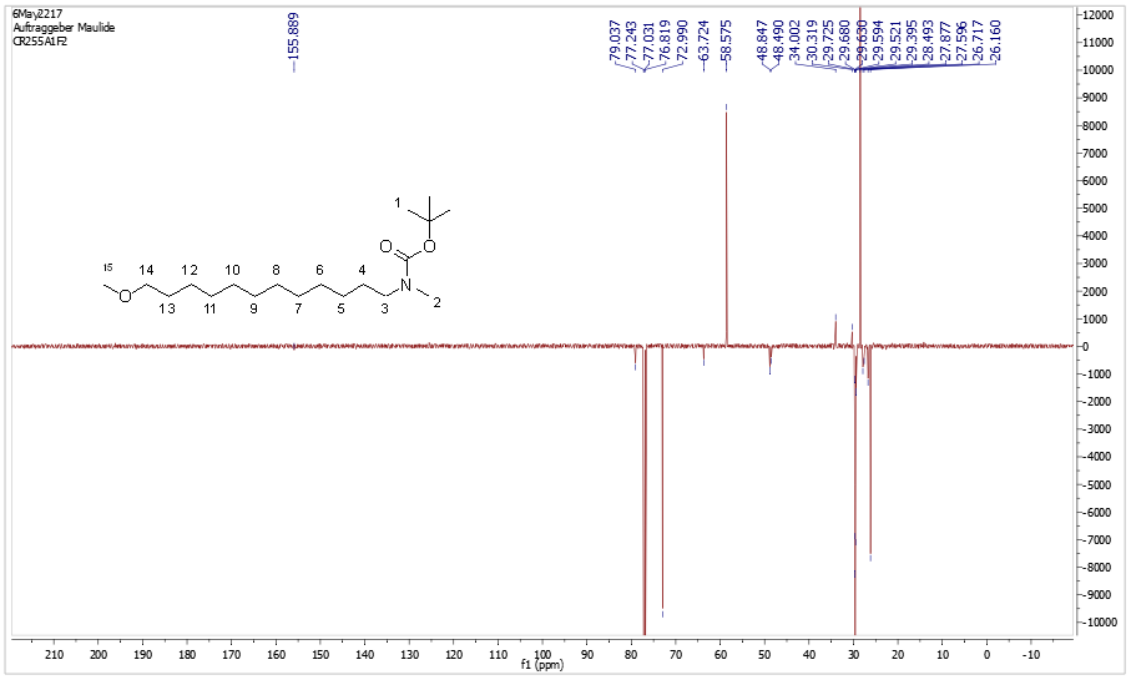

Figure 41 - ^{13}C NMR spectra of *tert*-Butyl (13-methoxytridecyl)(methyl)carbamate (5.14).

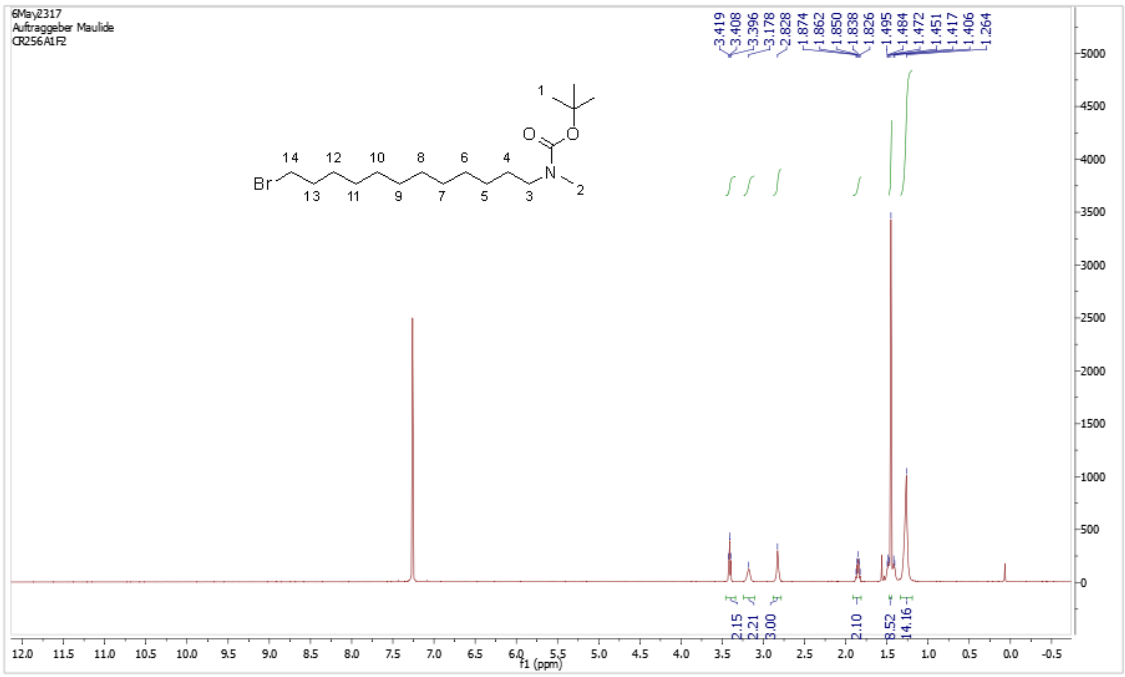

Figure 42 - ^{1}H NMR spectra of *tert*-Butyl (13-bromotridecyl)(methyl)carbamate (5.18).

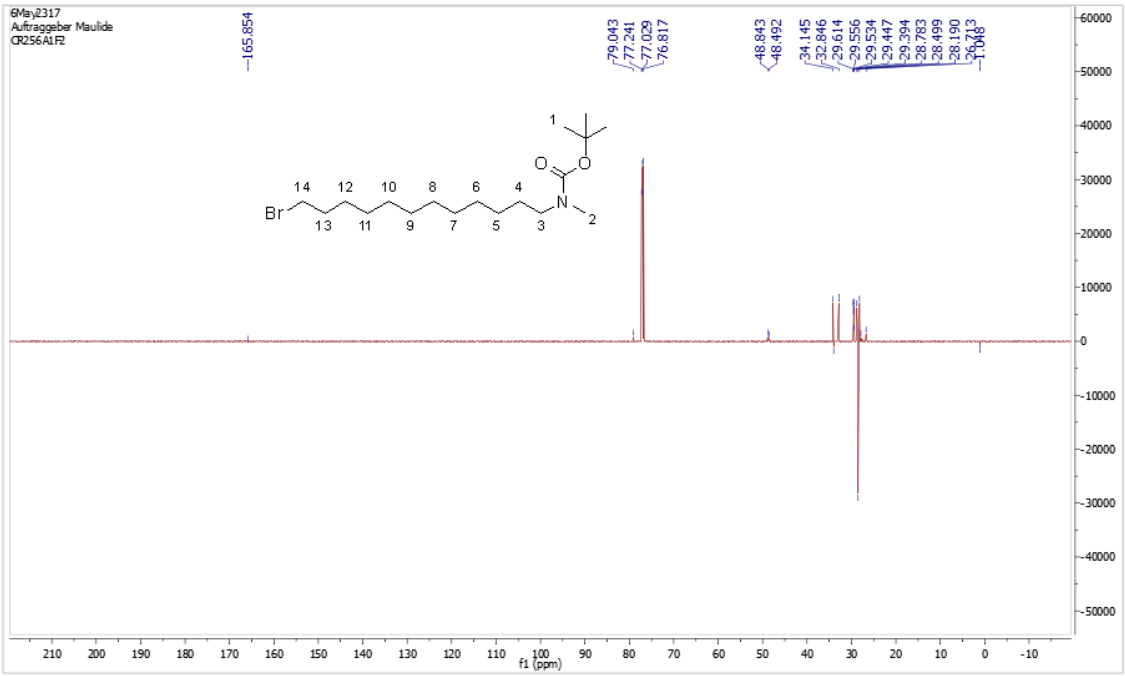

Figure 43 - ^{13}C NMR spectra of *tert*-Butyl (13-bromotridecyl)(methyl)carbamate (5.18).

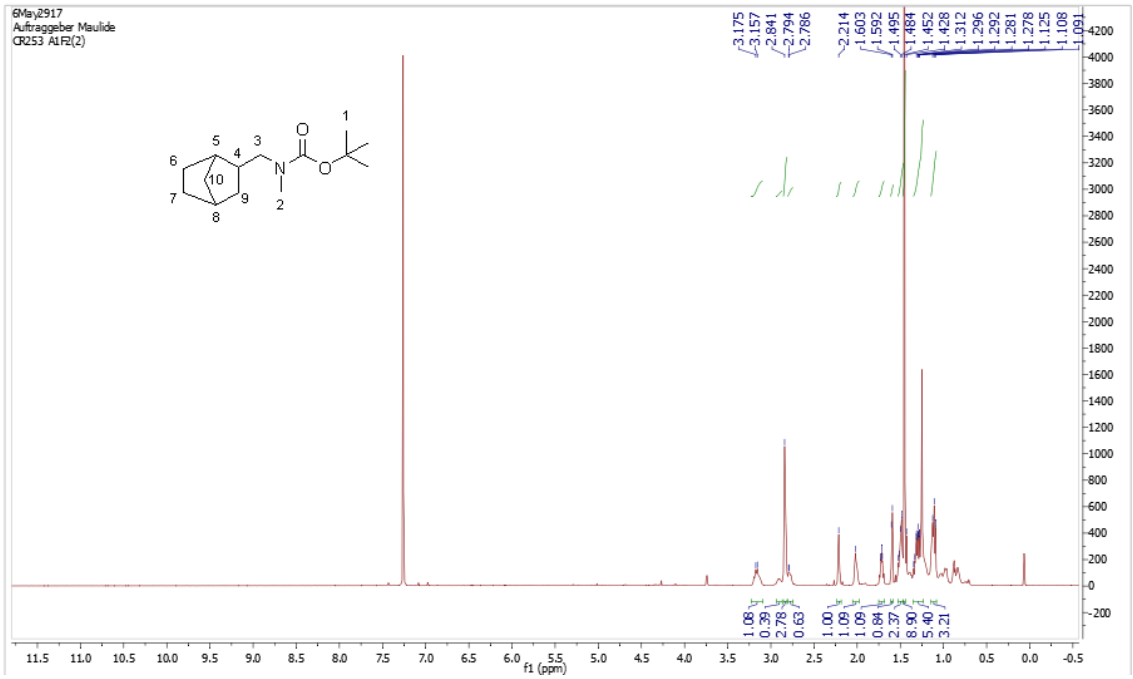

Figure 44 - ^{1}H NMR spectra of 6-*tert*-Butyl (bicyclo[2.2.1]heptan-2-ylmethyl)(methyl)carbamate (12.2).

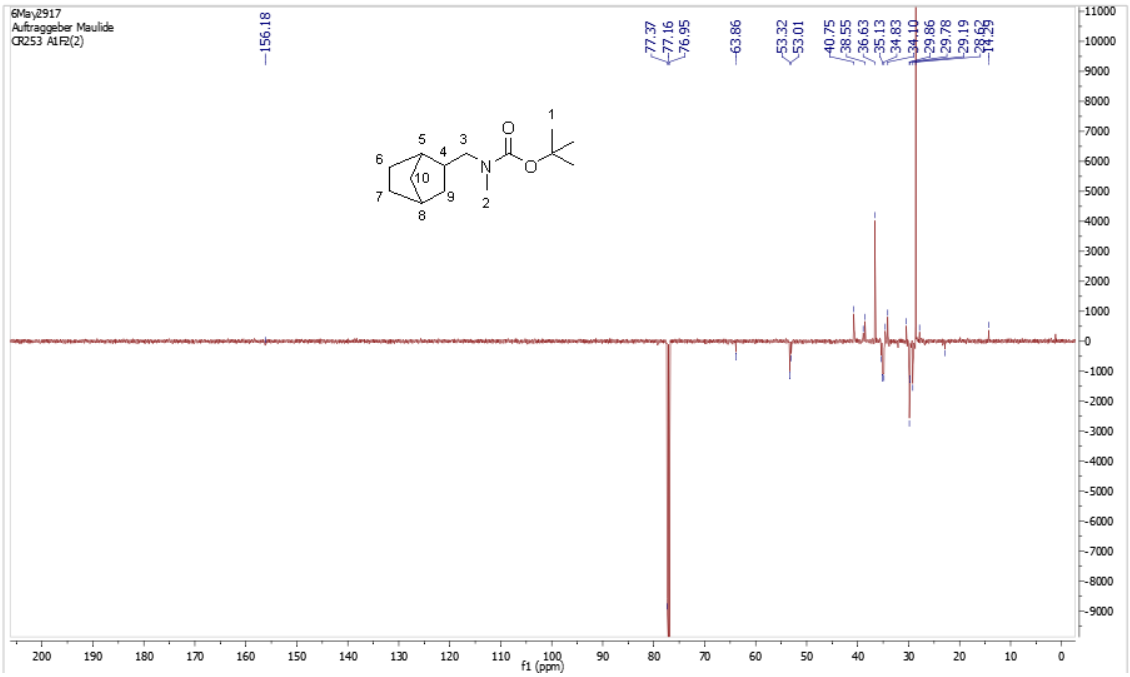

Figure 45 - ^{13}C NMR spectra of 6-*tert*-Butyl (bicyclo[2.2.1]heptan-2-ylmethyl)(methyl)carbamate (12.2).

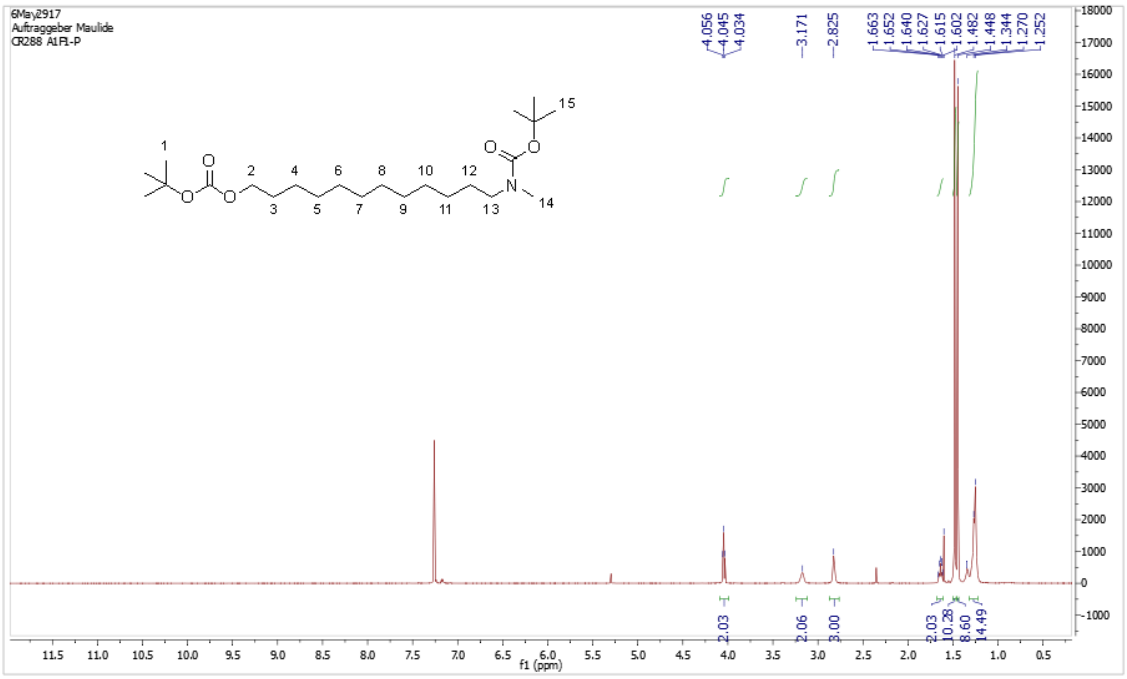

Figure 46 - ^{1}H NMR spectra of *tert*-butyl (13-((tert-butoxycarbonyl)oxy)tridecyl)(methyl)carbamate (5.19).

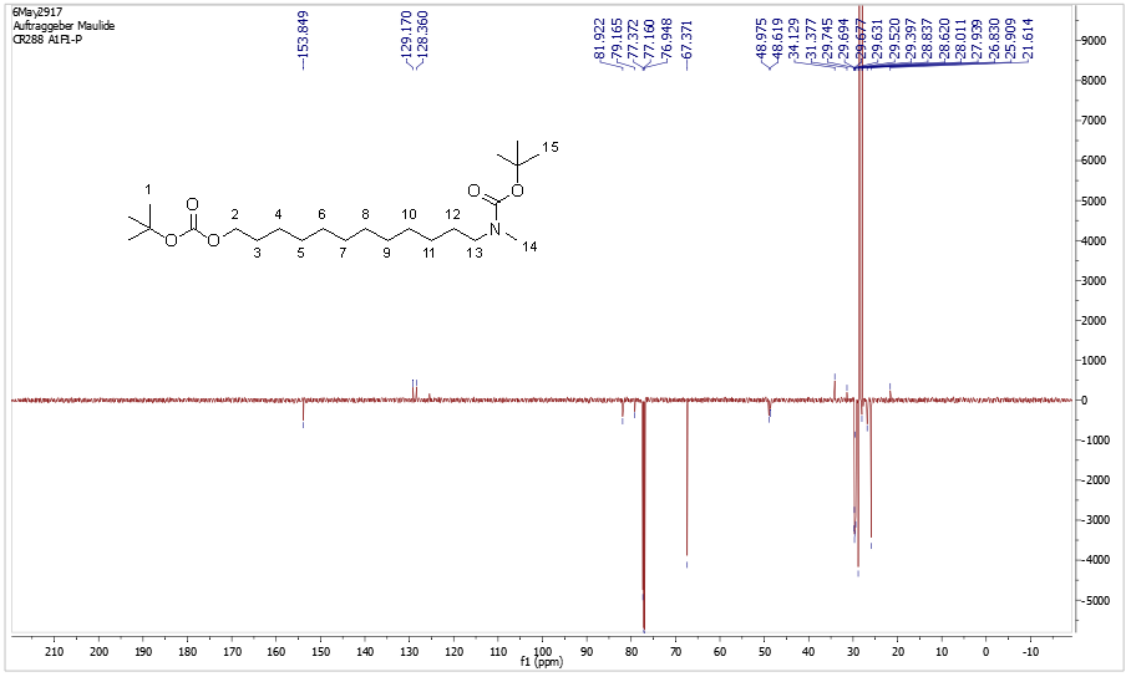

Figure 47 - 13C NMR spectra of tert-butyl (13-((*tert*-butoxycarbonyl)oxy)tridecyl)(methyl)carbamate(5.19).

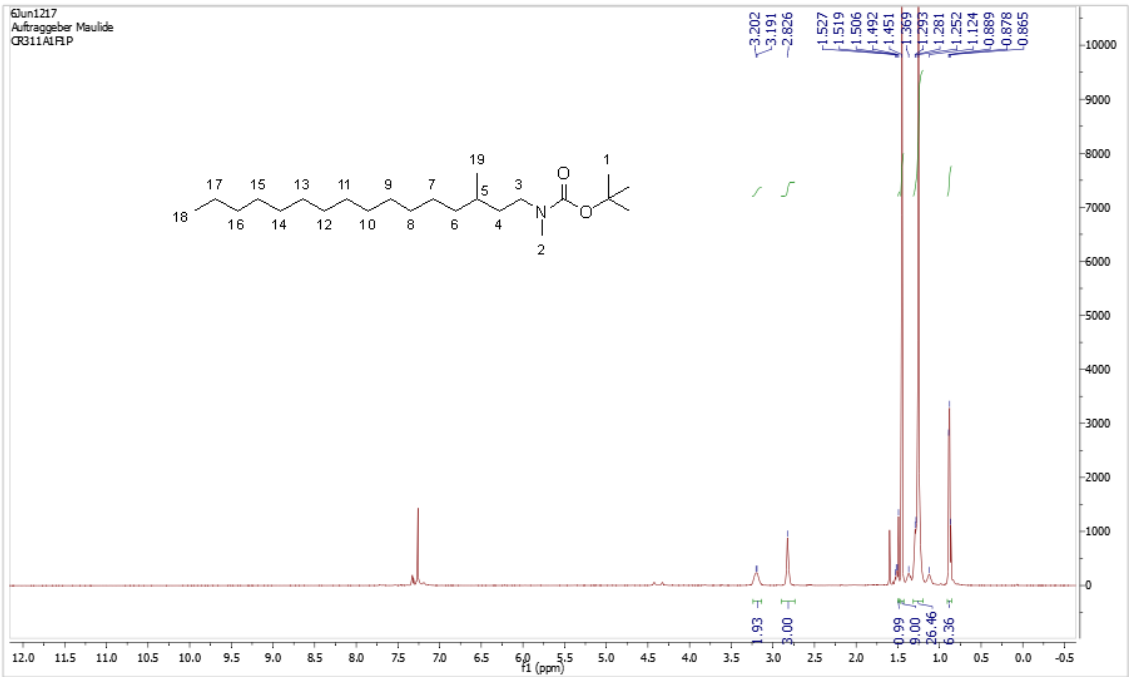

Figure 48 - ^{1}H NMR spectra of *tert*-Butyl methyl(3-methylhexadecyl)carbamate (5.8).

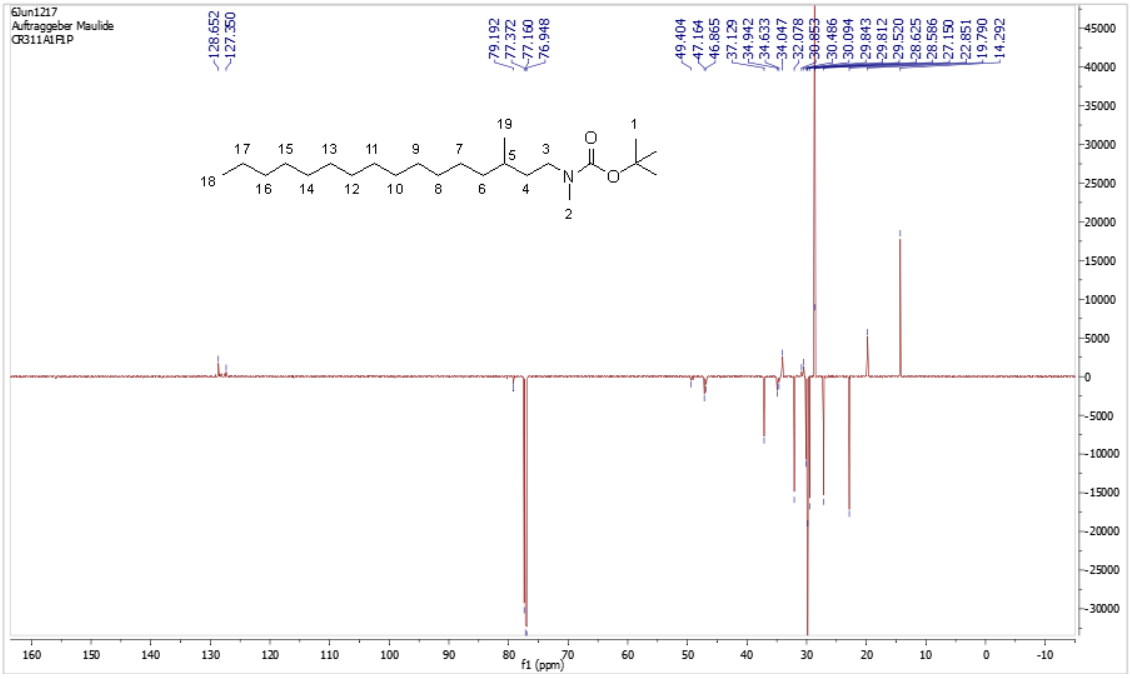

Figure 49 - ^{13}C NMR spectra of *tert*-Butyl methyl(3-methylhexadecyl)carbamate (5.8).

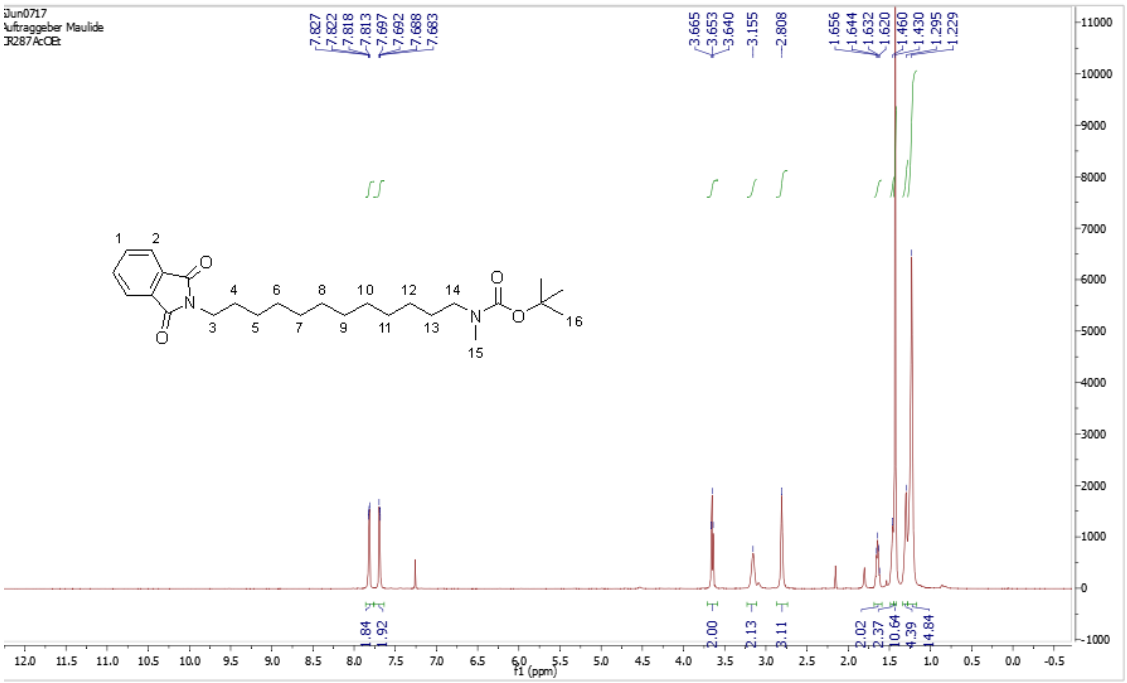

Figure 50 - ^{1}H NMR spectra of *tert*-Butyl (12-(1,3-dioxoisoindolin-2-yl)dodecyl)(methyl)carbamate (5.16).

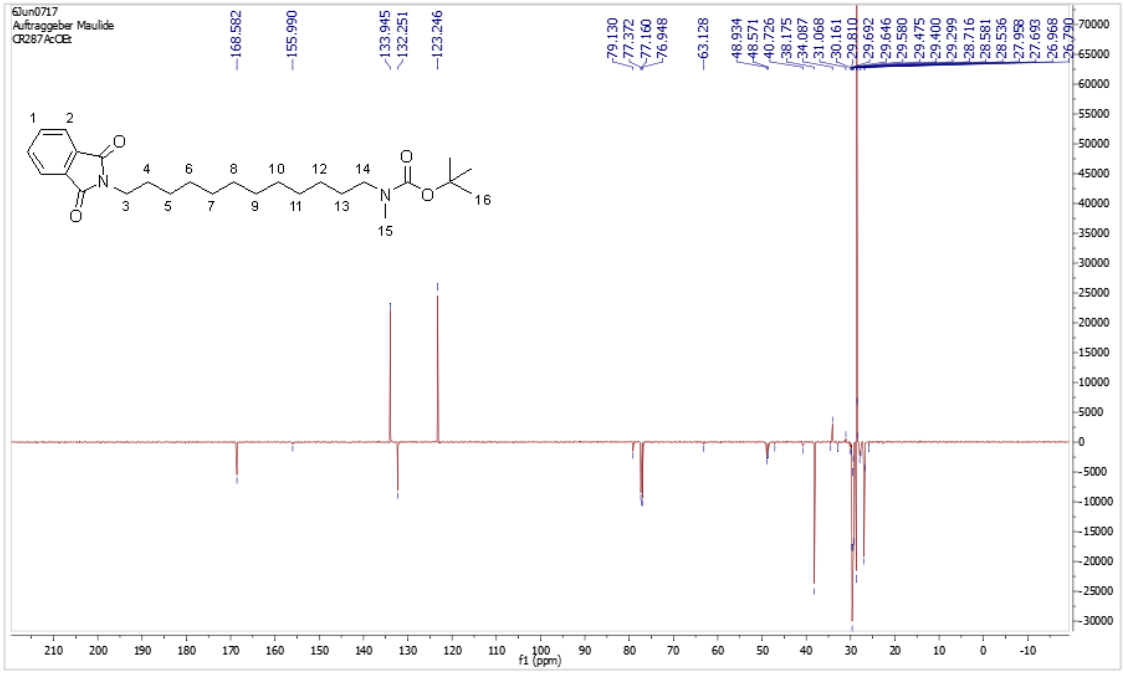

Figure 51 - ^{13}C NMR spectra of *tert*-Butyl (12-(1,3-dioxoisoindolin-2-yl)dodecyl)(methyl)carbamate (5.16).

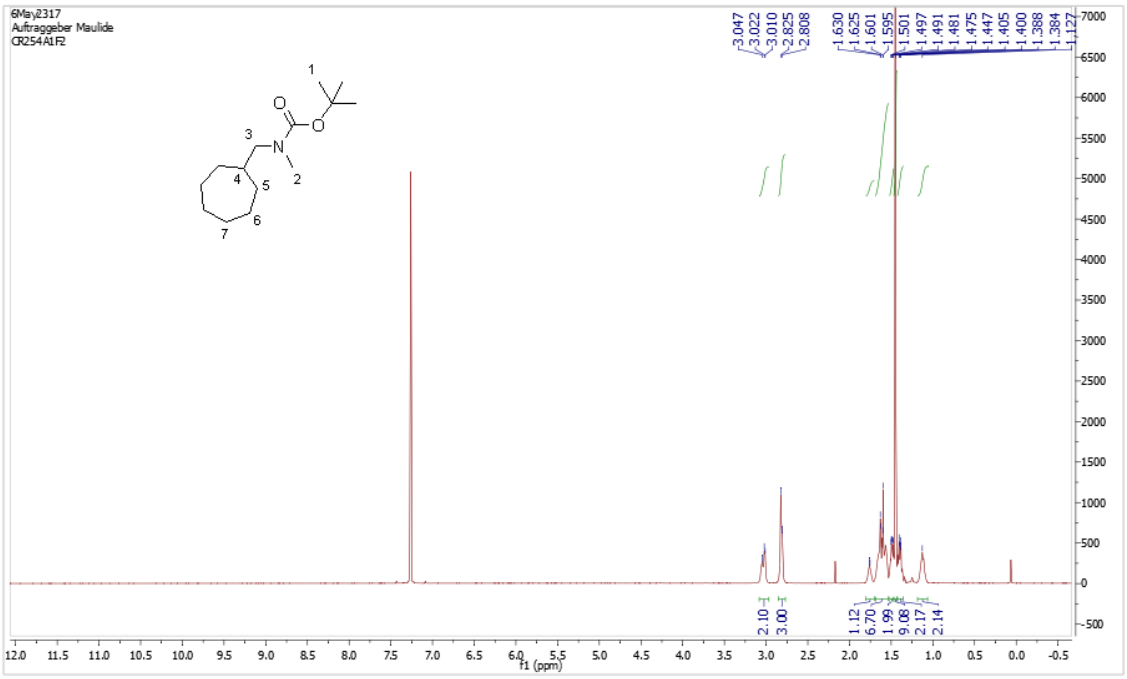

Figure 52 - ^{1}H NMR spectra of *tert*-Butyl (cycloheptylmethyl)(methyl)carbamate (5.12).

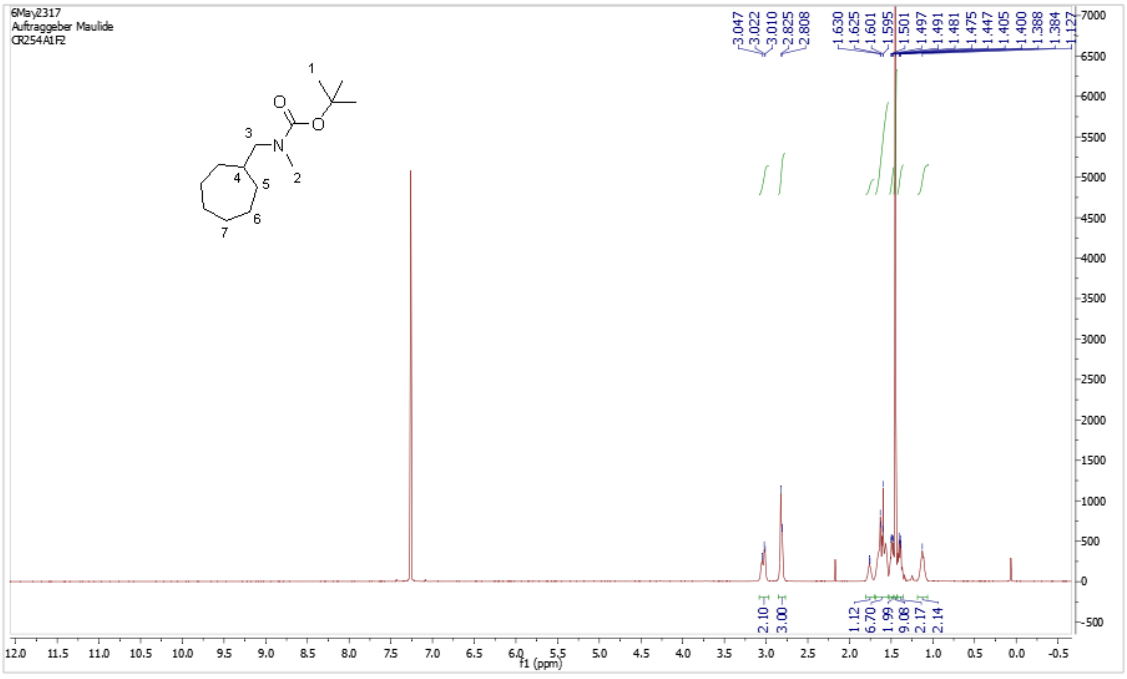

Figure 53 - ^{13}C NMR spectra of *tert*-Butyl (cycloheptylmethyl)(methyl)carbamate (5.12).

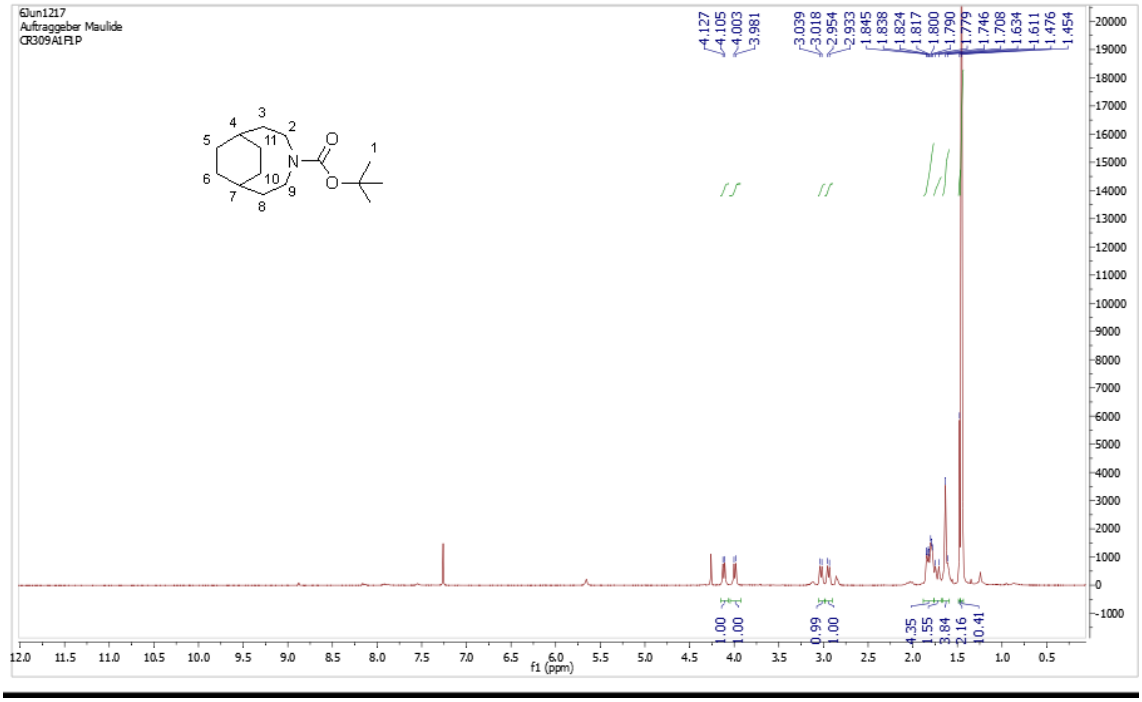

Figure 54 - ^{1}H NMR spectra of *tert*-butyl 4-azabicyclo[5.2.2]undecane-4-carboxylate (12.2).

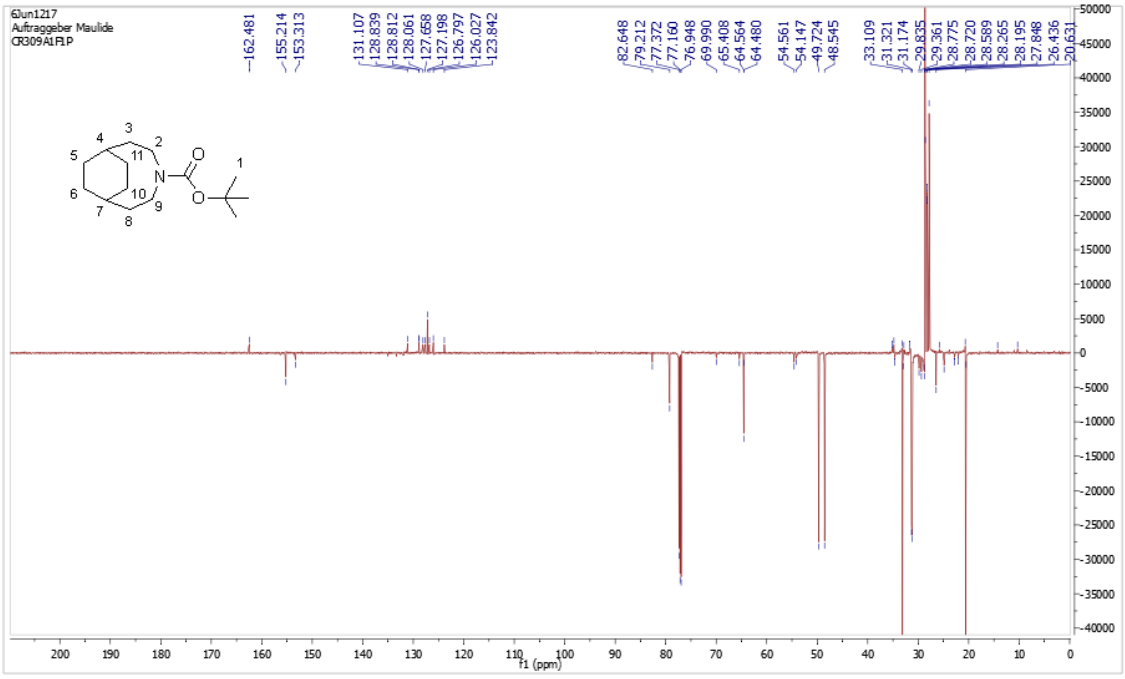

Figure 55 - ^{13}C NMR spectra of *tert*-butyl 4-azabicyclo[5.2.2]undecane-4-carboxylate (12.2).

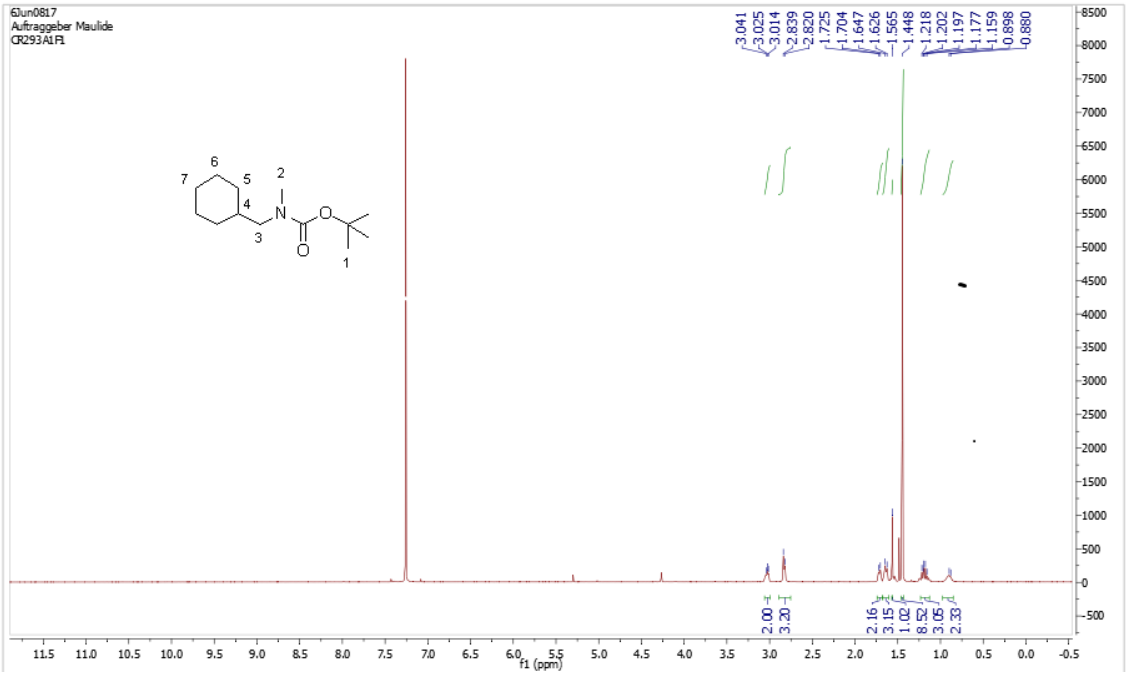

Figure 56 - ^{1}H NMR spectra of *tert*-butyl (cyclohexylmethyl)(methyl)carbamate (5.4).

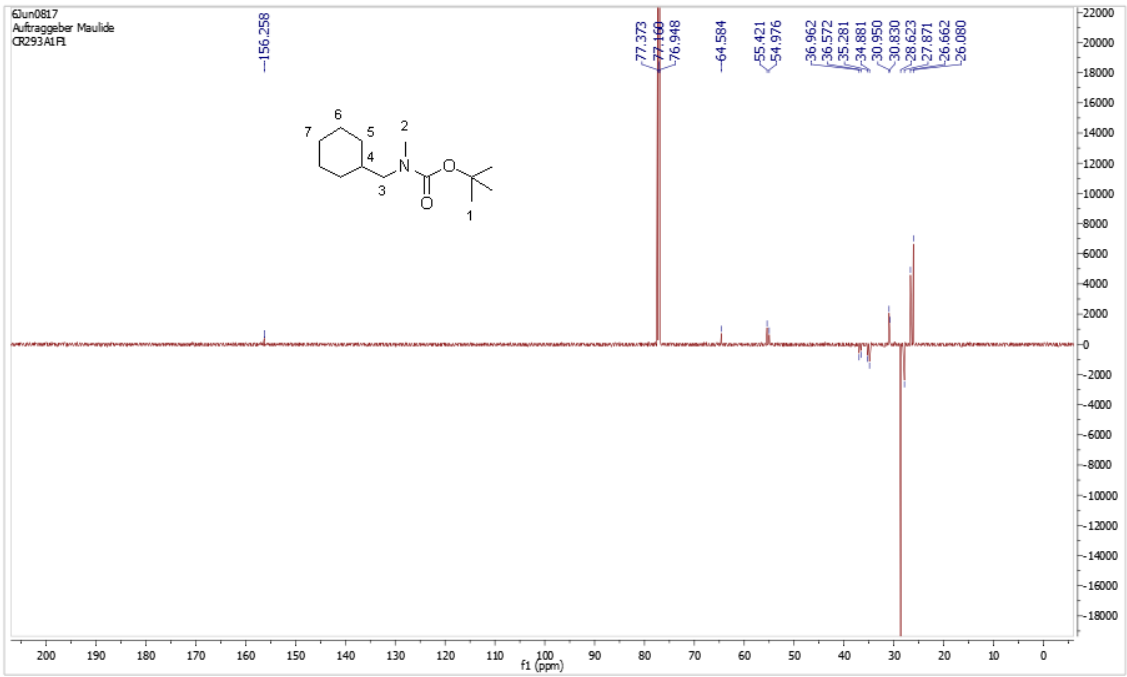

Figure 57 - ^{13}C NMR spectra of *tert*-butyl (cyclohexylmethyl)(methyl)carbamate (5.4).

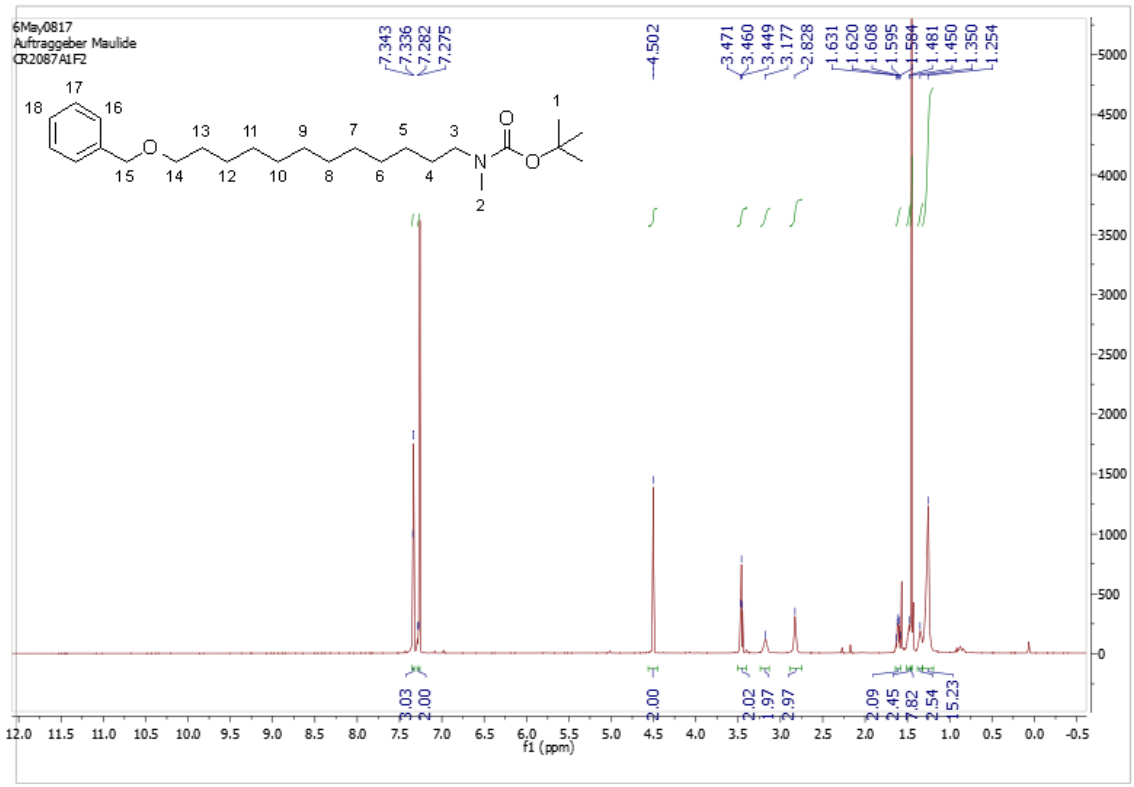

Figure 58 - ^{1}H NMR spectra of *tert*-Butyl (12-(benzyloxy)dodecyl)(methyl)carbamate (5.21).

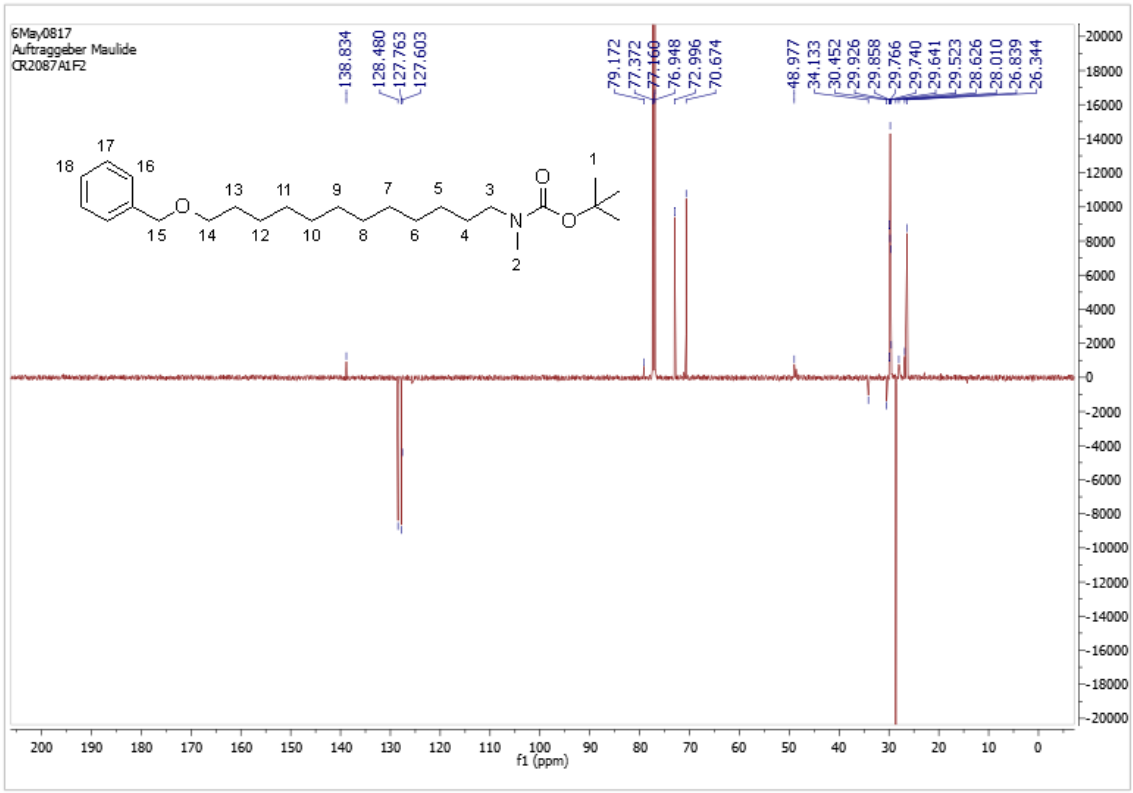

Figure 59 - ^{13}C NMR spectra of *tert*-Butyl (12-(benzyloxy)dodecyl)(methyl)carbamate (5.21).

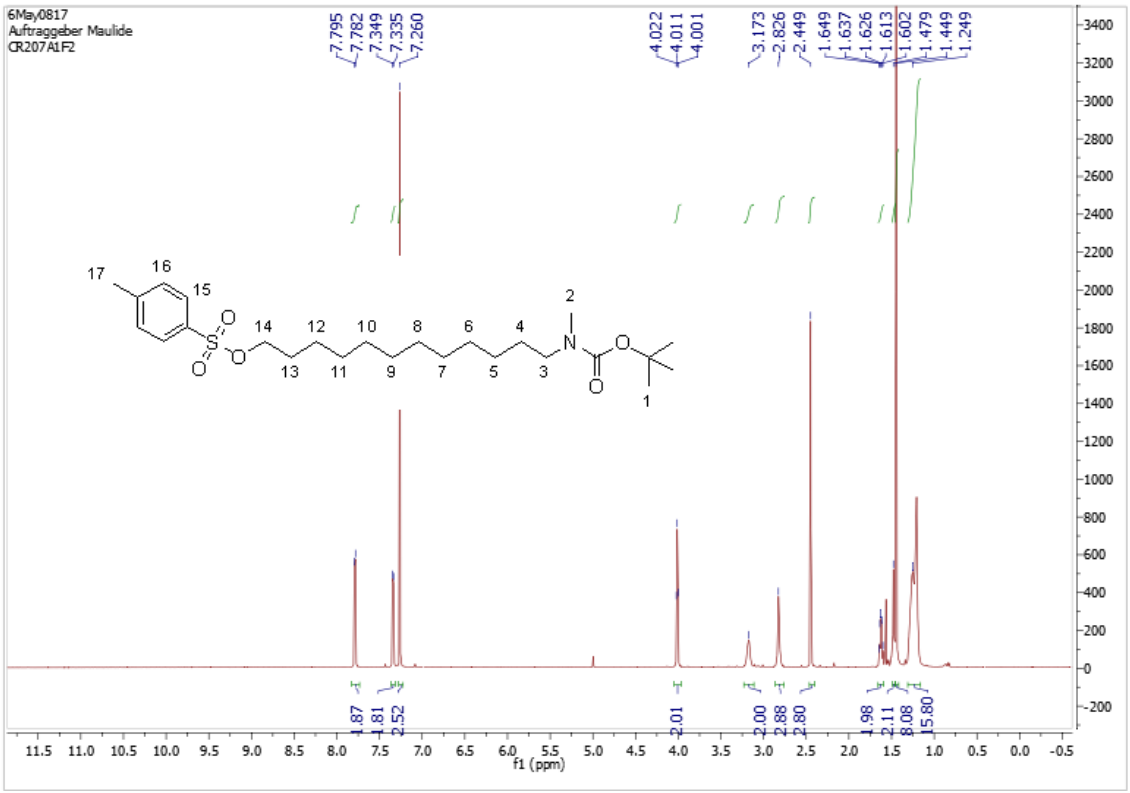

Figure 60 - ^{1}H NMR spectra of 12-((*tert*-Butoxycarbonyl)(methyl)amino)dodecyl 4-methylbenzenesulfonate (5.17).

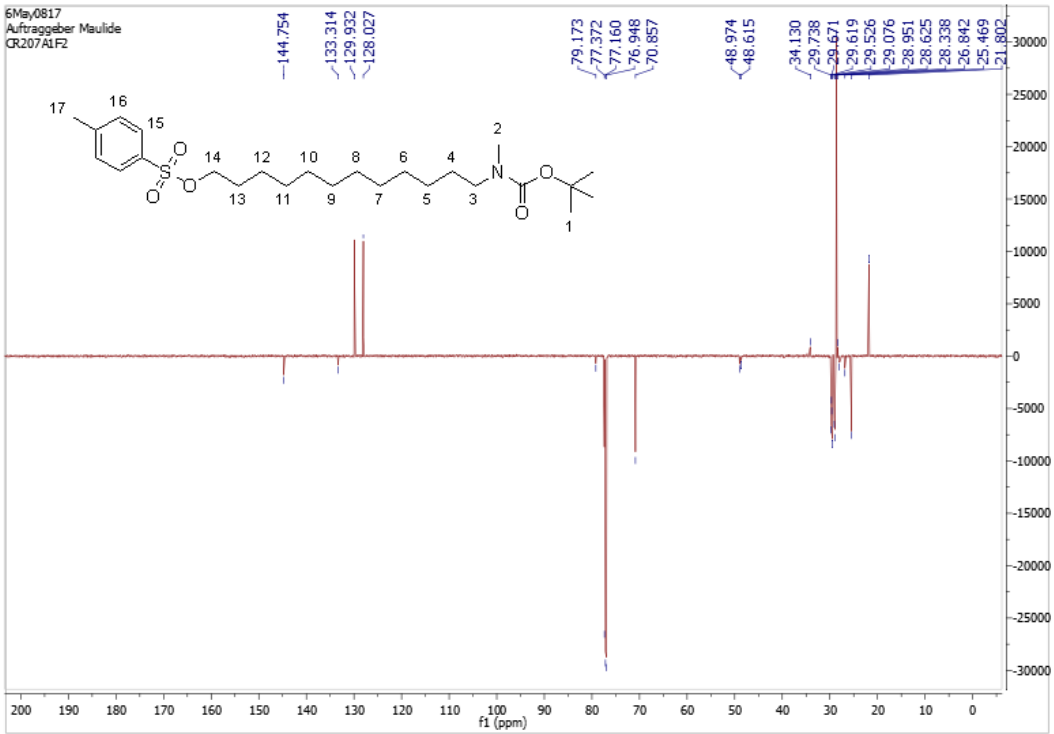

Figure 61 - ^{13}C NMR spectra of 12-((*tert*-Butoxycarbonyl)(methyl)amino)dodecyl 4-methylbenzenesulfonate (5.17).

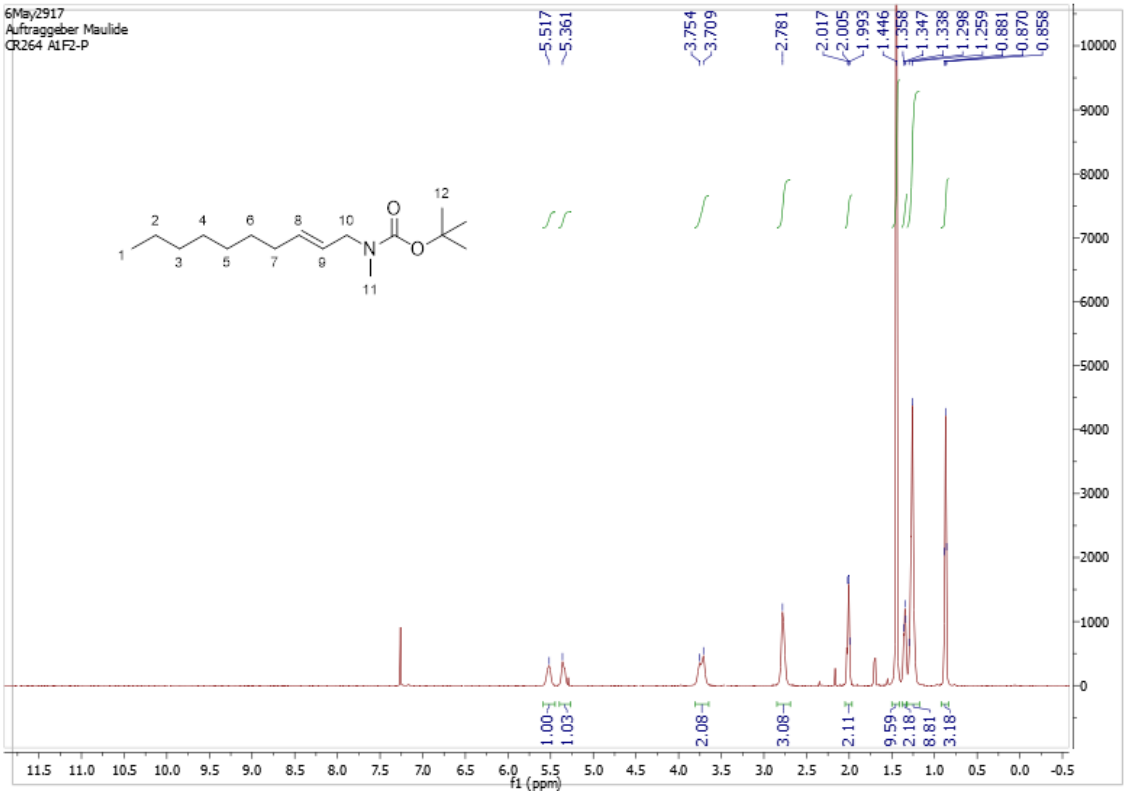

Figure 62 - [1]H NMR spectra of *tert*-Butyl (E)-dec-2-en-1-yl(methyl)carbamate(9.2).

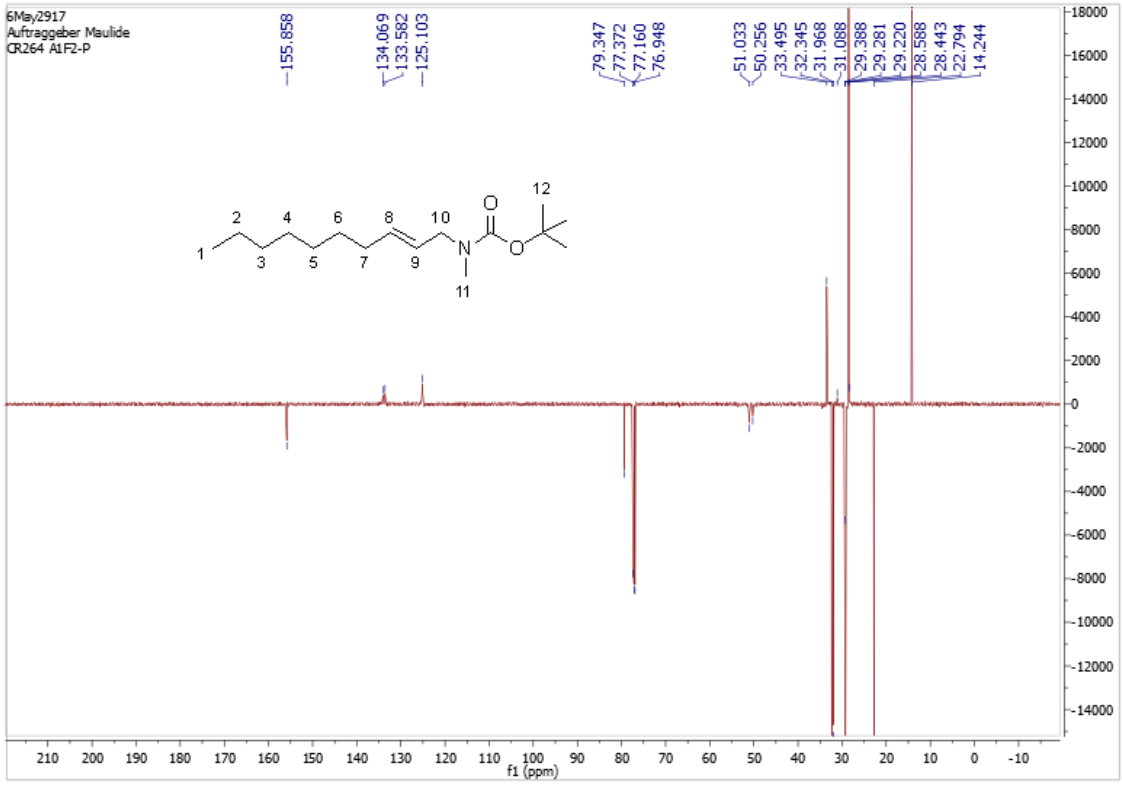

Figure 63 - [13]C NMR spectra of *tert*-Butyl (E)-dec-2-en-1-yl(methyl)carbamate(9.2).

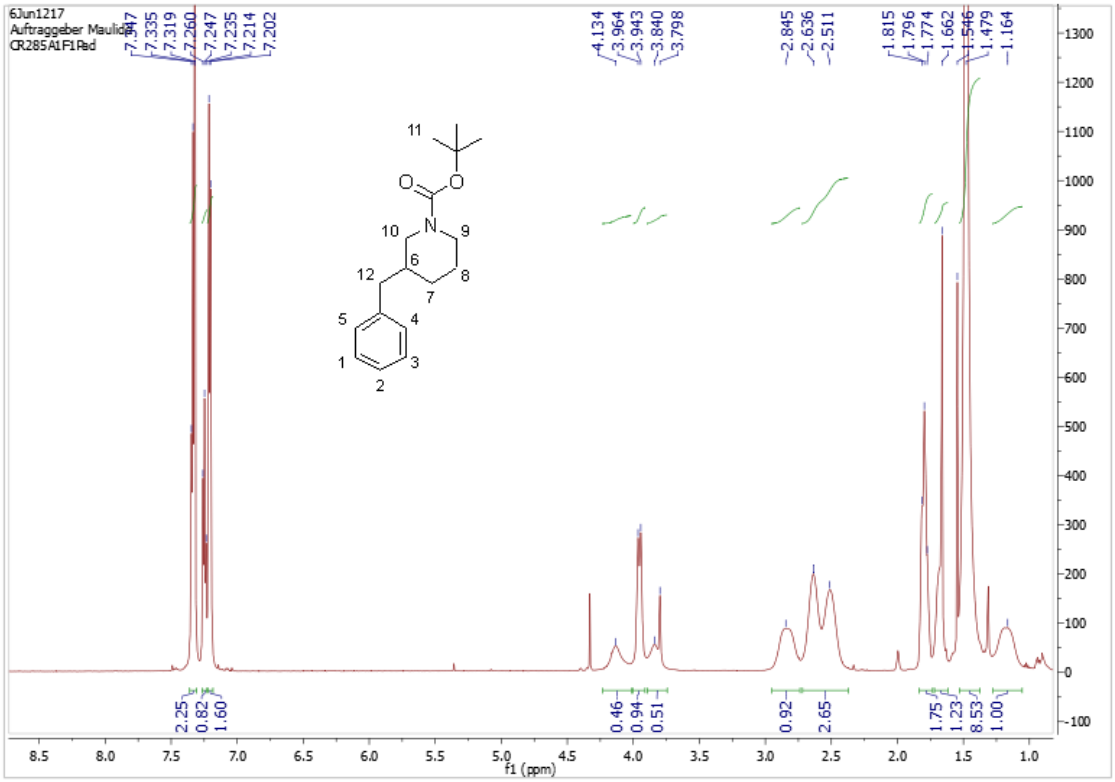

Figure 64 - [1]H NMR of spectra *tert*-Butyl 3-phenylpiperidine-1-carboxylate(12.1).

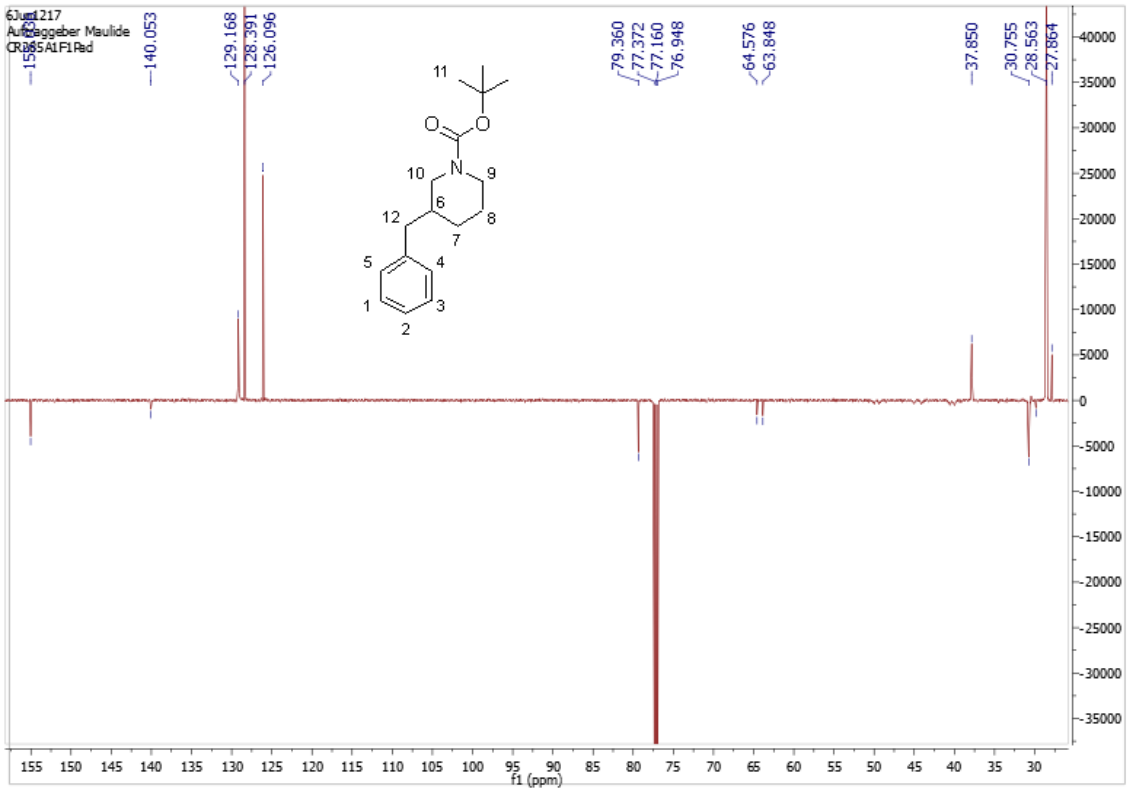

Figure 65 - [13]C NMR of spectra *tert*-Butyl 3-phenylpiperidine-1-carboxylate(12.1).

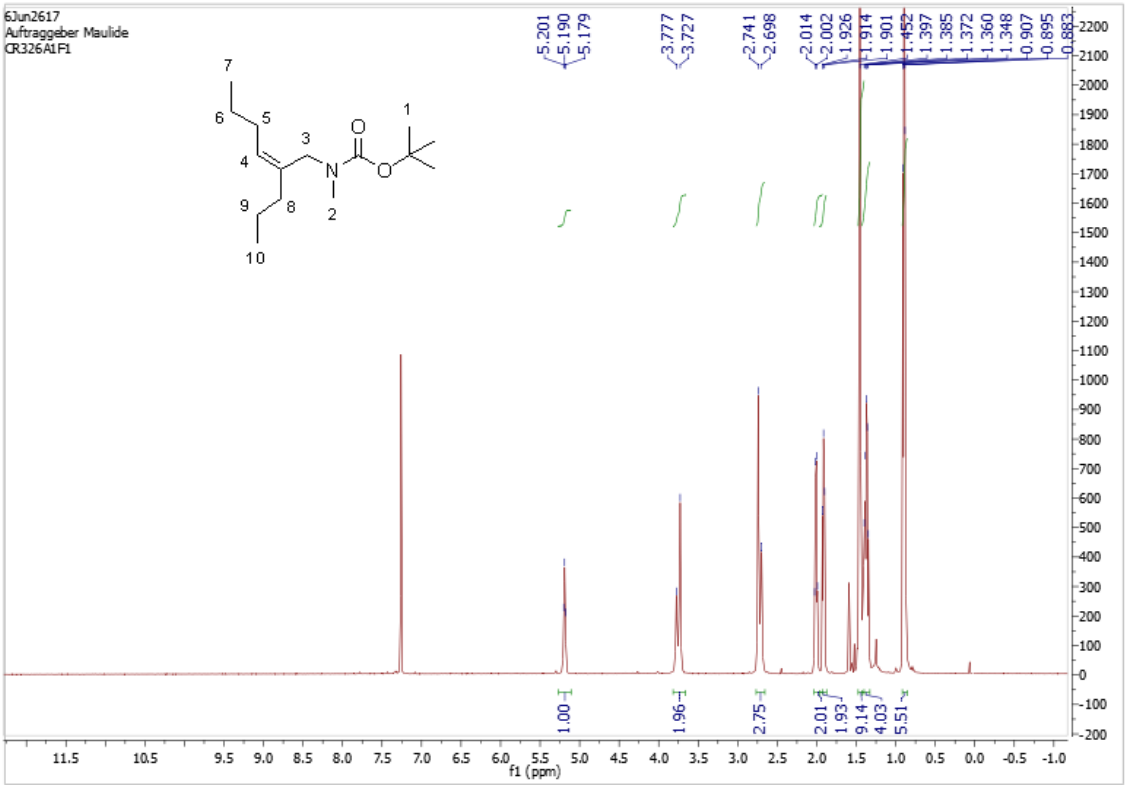

Figure 66 - [1]H NMR spectra of *tert*-Butyl (Z)-methyl(2-propylhex-2-en-1-yl)carbamate (9.1).

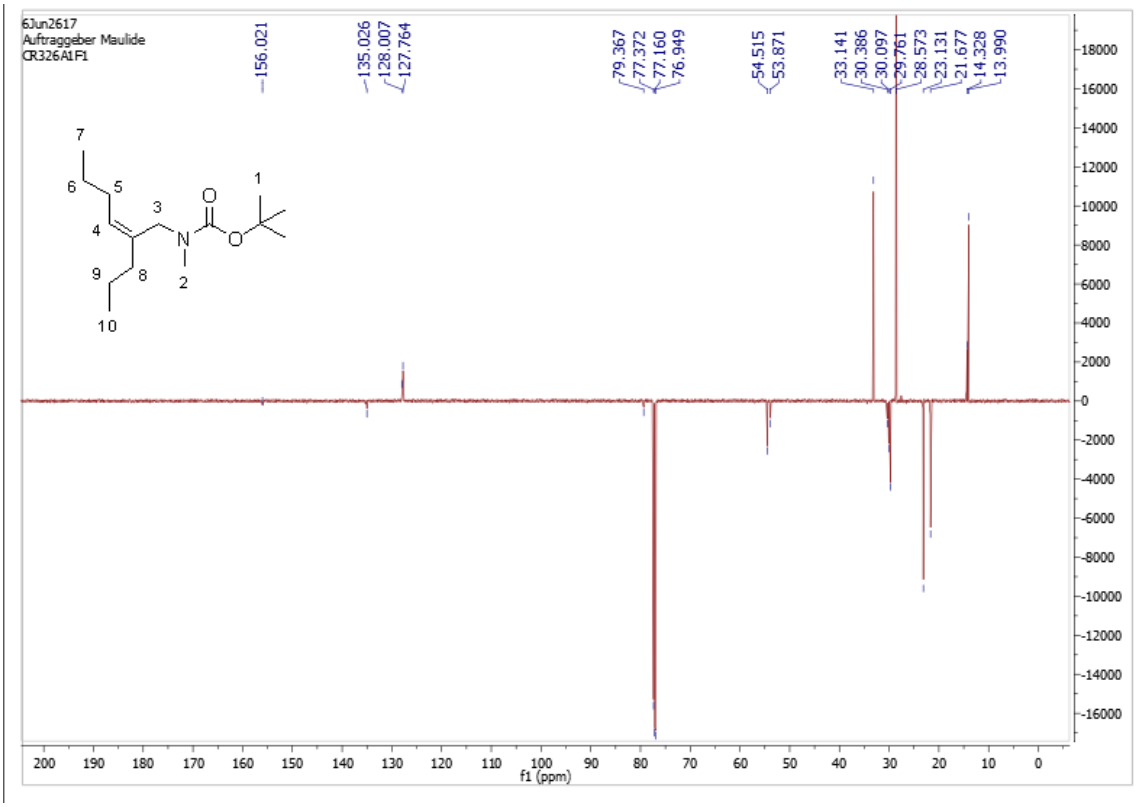

Figure 67 - [13]C NMR spectra of *tert*-Butyl (Z)-methyl(2-propylhex-2-en-1-yl)carbamate (9.1).

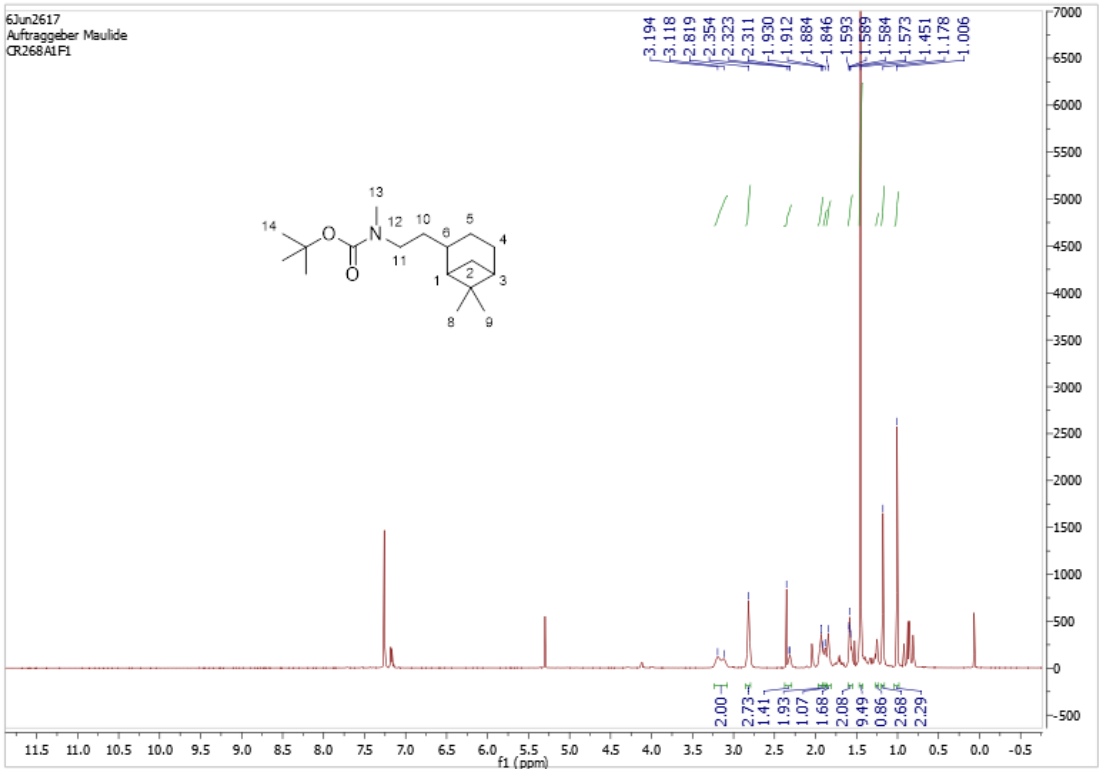

Figure 68 - ^{1}H NMR spectra of *tert*-Butyl (2-(6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethyl)(methyl)carbamate (5.11).

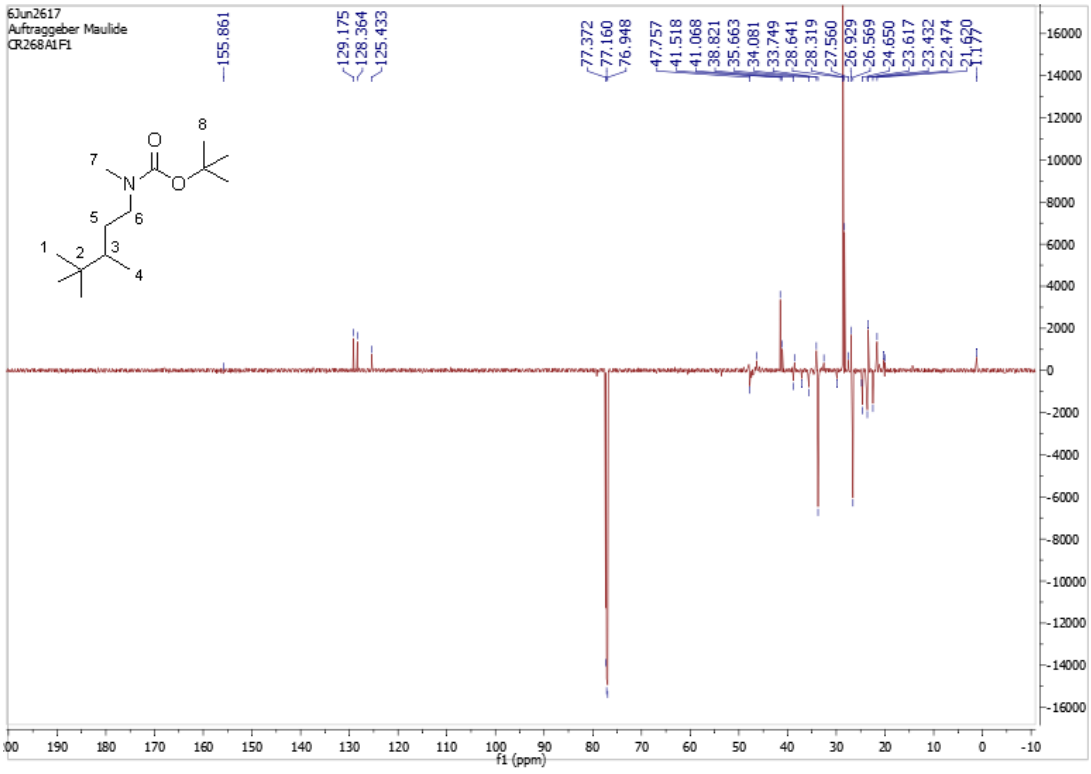

Figure 69 - ^{13}C NMR spectra of *tert*-Butyl (2-(6,6-dimethylbicyclo[3.1.1]heptan-2-yl)ethyl)(methyl)carbamate (5.11).

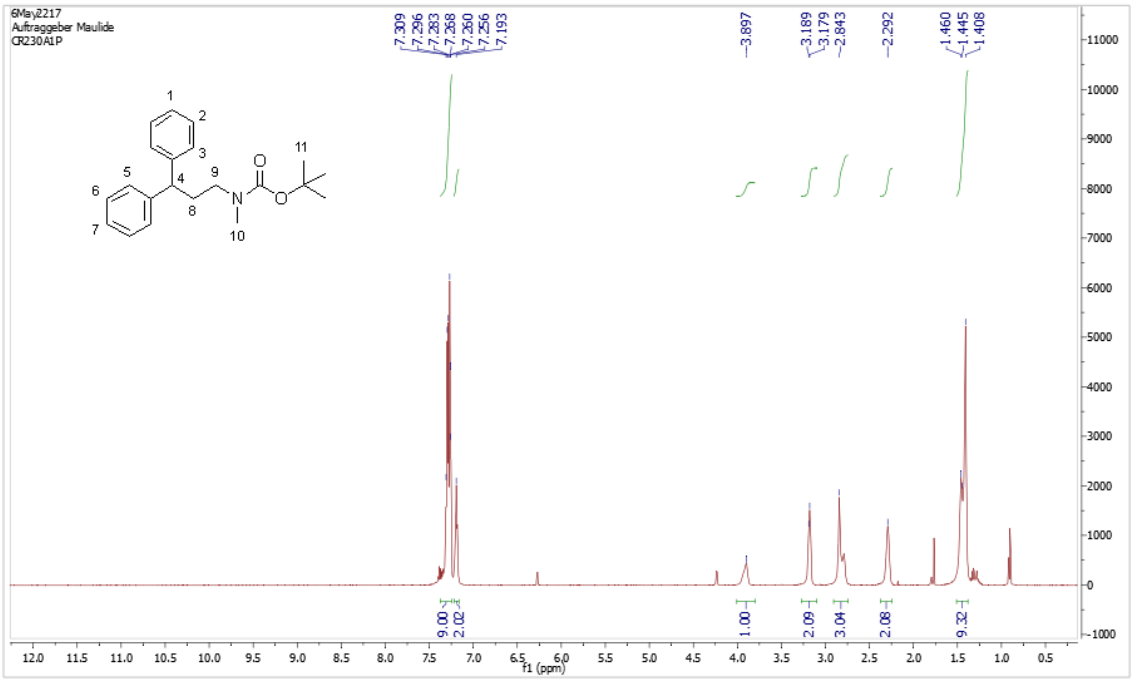

Figure 70 - ^{1}H NMR spectra of *tert*-Butyl (3,3-diphenylpropyl)(methyl)carbamate(5.9).

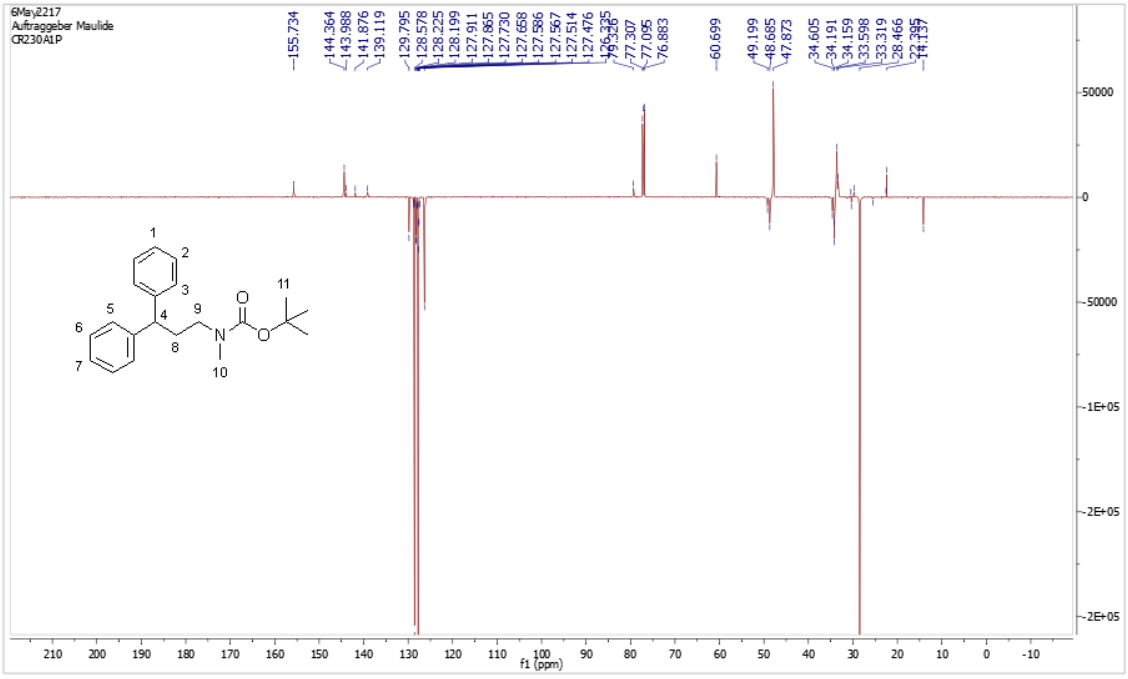

Figure 71 - ^{13}C NMR spectra of *tert*-Butyl (3,3-diphenylpropyl)(methyl)carbamate(5.9).

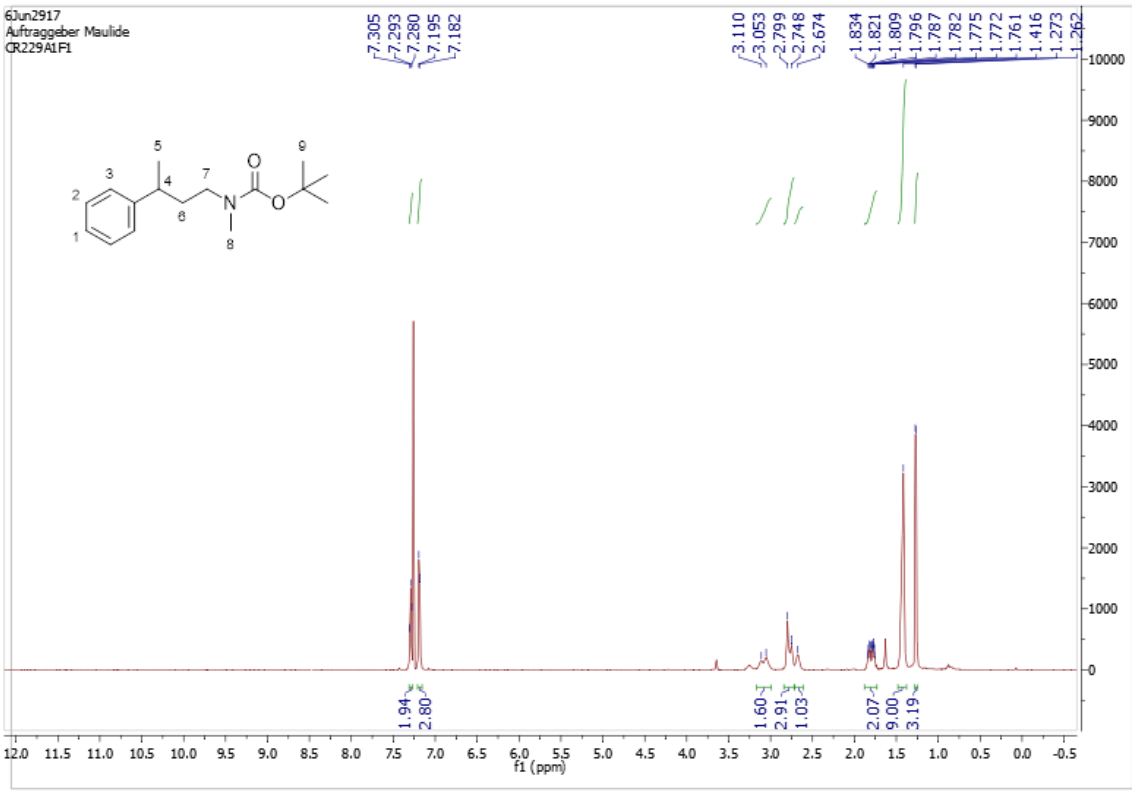

Figure 72 - ^{1}H NMR spectra of *tert*-Butyl methyl(3-phenylbutyl)carbamate(5.10).

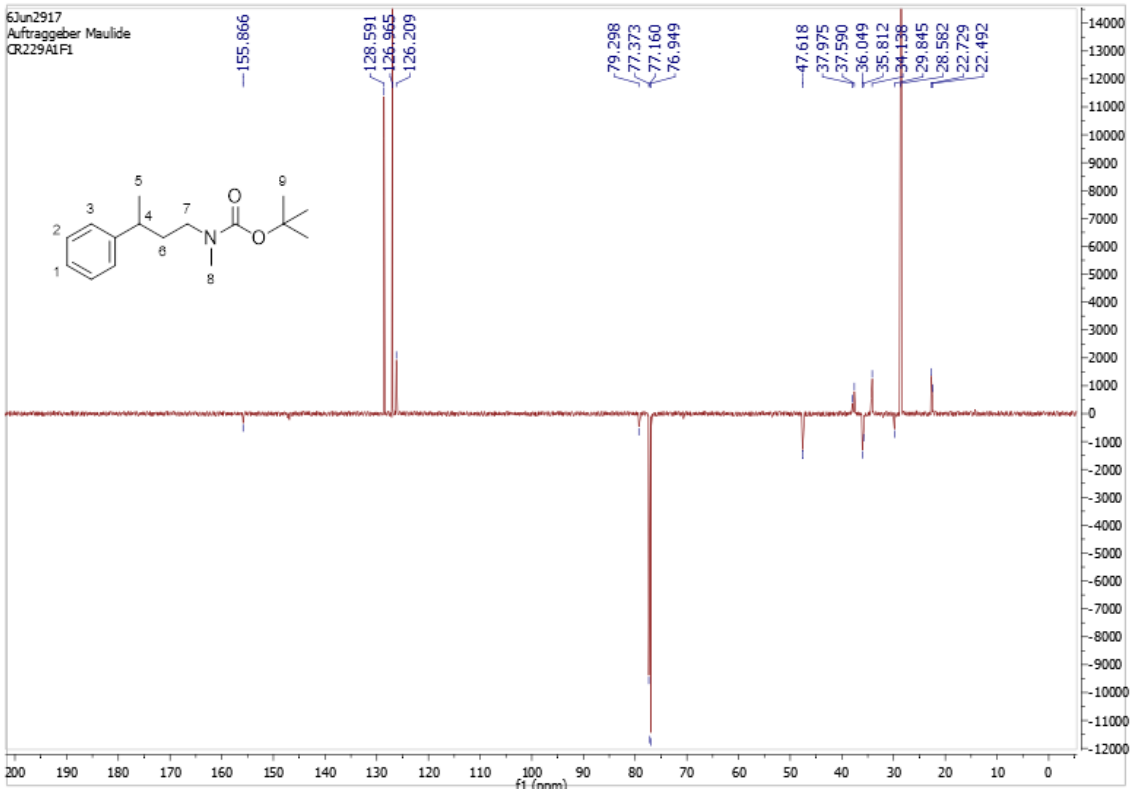

Figure 73 - ^{13}C NMR spectra of *tert*-Butyl methyl(3-phenylbutyl)carbamate(5.10).

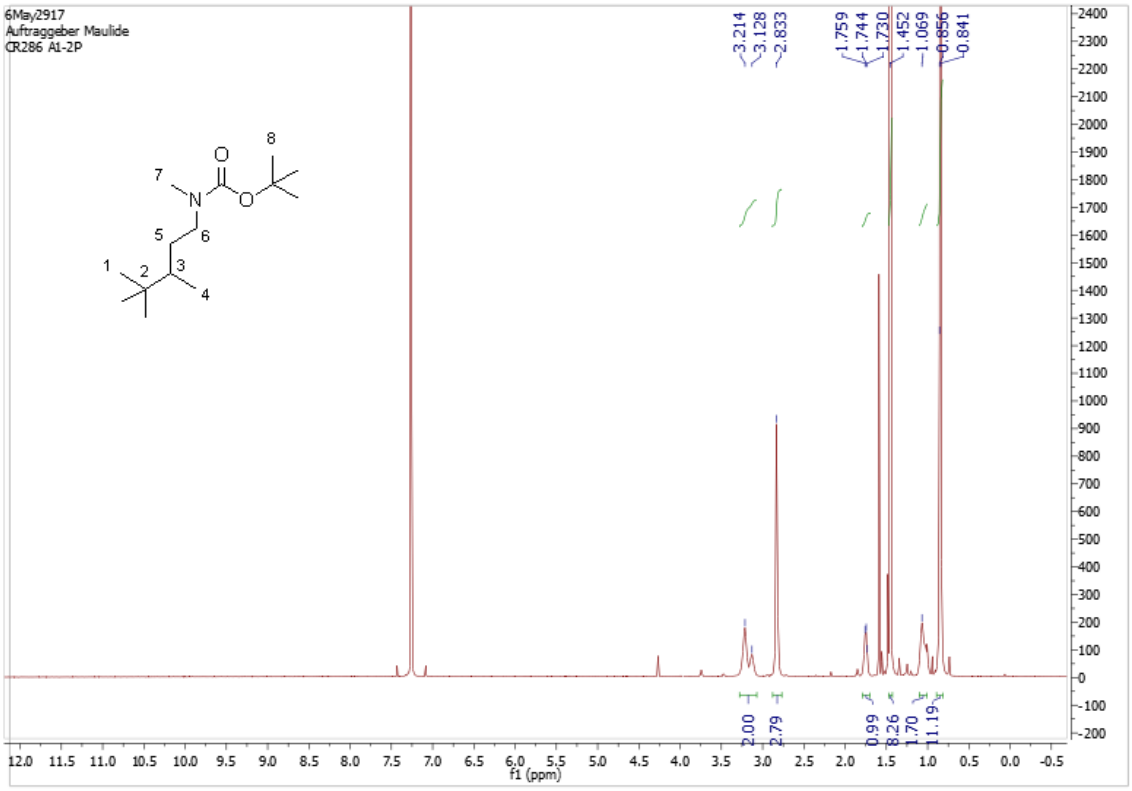

Figure 74 - ^{1}H NMR spectra of *tert*-Butyl methyl(3,4,4-trimethylpentyl)carbamate(5.4).

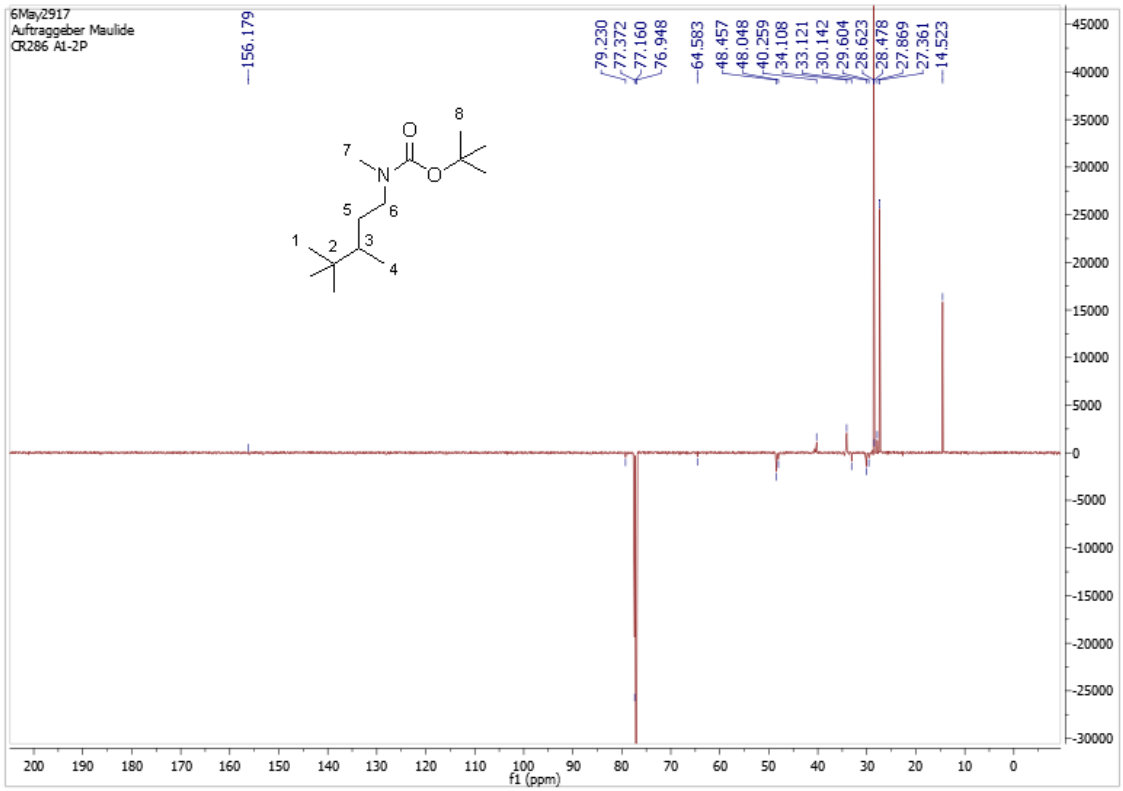

Figure 75 - ^{13}C NMR spectra of *tert*-Butyl methyl(3,4,4-trimethylpentyl)carbamate(5.4).

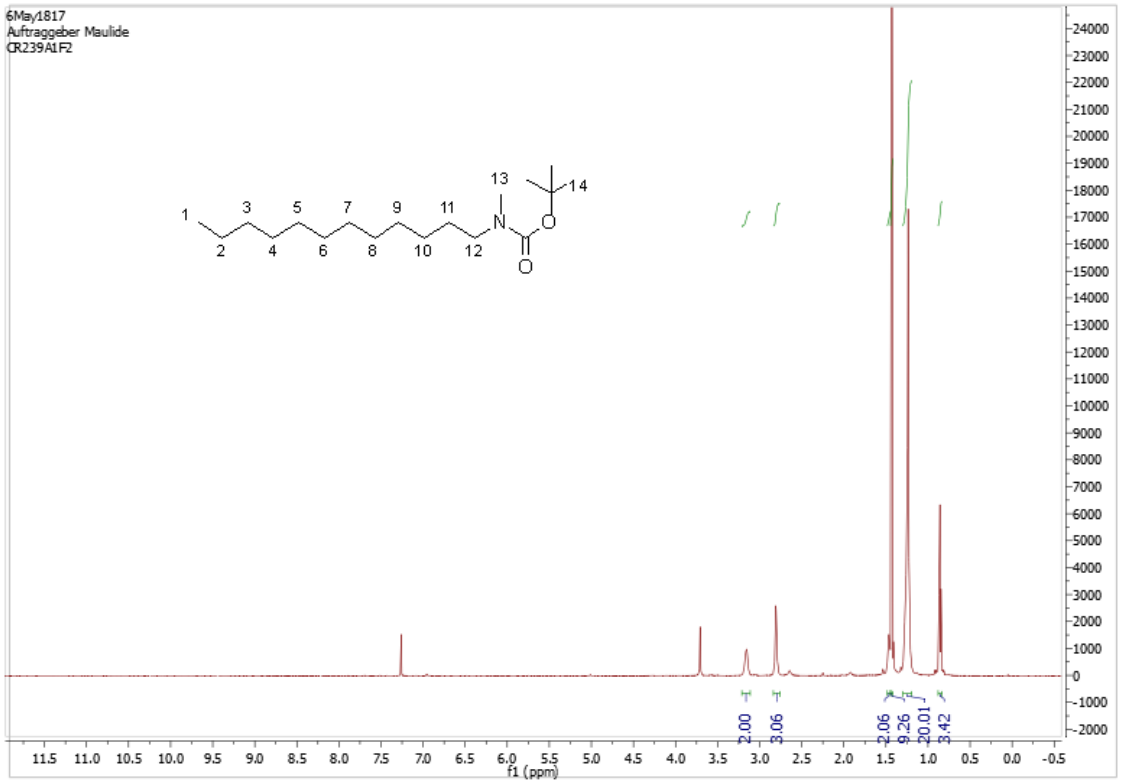

Figure 76 - ^{1}H NMR spectra of tert-Butyl dodecyl(methyl)carbamate(5.1).

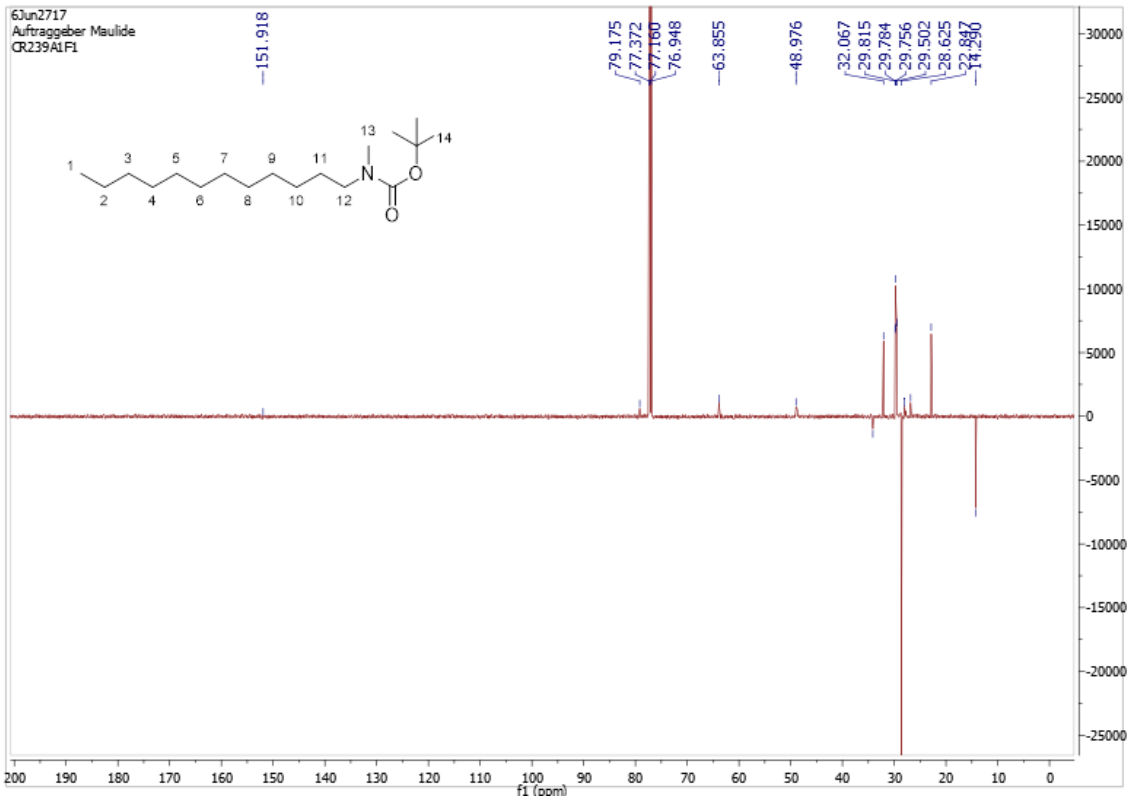

Figure 77 - ^{13}C NMR spectra of *tert*-Butyl dodecyl(methyl)carbamate(5.1).

Part two - Synthetic studies on a novel celastrol- fluorescent probe for in vivo imaging

Scheme Index

Table Index

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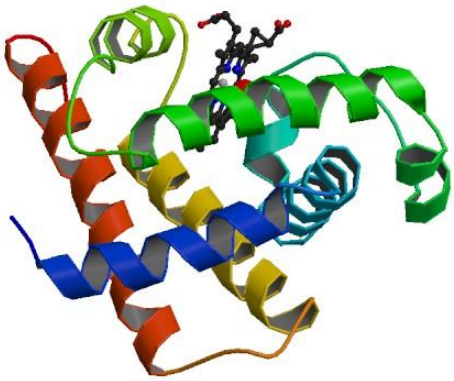
I-Introduction

In the past decades we have seen an astonishing development in the pharmaceutical industry that accompanied and helped to flourish the average life expectancy and, more importantly, happiness. Different studies also sustain these acts, which are not only linked to one another but are also commutatively connected between them [1] .

The large investment made by pharmaceutical companies in research is essential in the pursuit of new techniques and pharmaceuticals that might help solve some of the many persisting problems in today's society. 19 out of 20 experimental developments fail, leading to high costs that should not, however, be considered waste[2].

By its own definition, disease is still a calamity that affects, and most likely will continue to affect, humankind as long as it exists. Since the beginning of human life many solutions emerged in order to solve this problem. The difficulty at the time was that there was almost no information besides empirical experiences. This continued for thousands of years with "treatments" being passed from generation to generation until science and technology caught up to higher standards.

It is a common misbelief that nowadays causes and effects of diseases are known in their totality. A fact that supports this statement is that the structure of enzymes, now understood as responsible for catalyzing numerous chemical reactions that occur in the organism every day, was only resolved in the late 1950s by X-Ray analysis [3],thus revealing that most secrets about our inherent biological operations are still relatively recent.



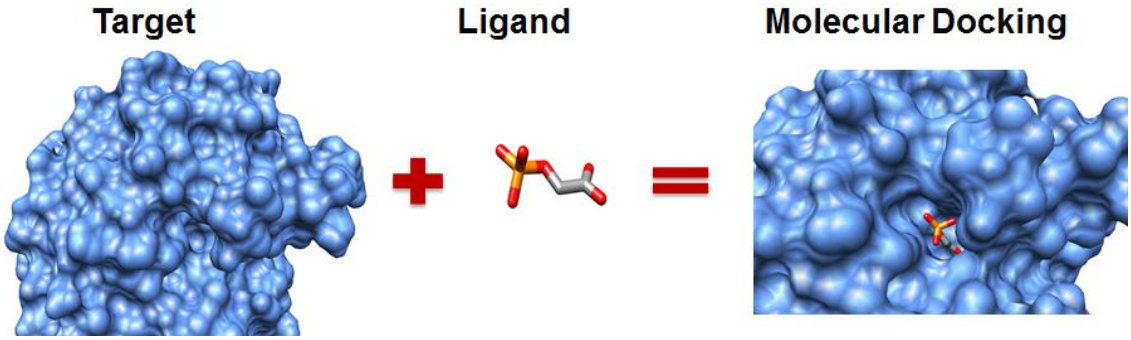
Scheme 41 – Molecular model of Myoglobin years after its first identification. Picture taken from PDB, id: 1MBN.

A large majority of the drugs used on the first part of the 20th century were compounds whose effects were only known due to previous experiences. This meant that any variations of commonly known illnesses led to major catastrophes – and that can be correlated by different epidemics throughout history. Although an almost exponential increase in knowledge occurred in the last few years there is still a lot to investigate. In relation to the manufacturing of therapeutic agents, there is a fairly recent realm of chemistry which has been getting a lot of attention - Medicinal chemistry.

Medicinal chemistry is essentially the meddling of several different areas, which range from bio chemistry to organic synthesis. In its core lie different notions which help to guide a directed synthesis of drugs – this means that instead of synthesizing a pharmaceutical based on its effects the design is guided by the interactions of the molecule with the active site of its target. It is evident that a lot of information is required in order for it to be successful. Structures of metabolites, cells and pathways the drug traveled were unknown for a long time and had to be discovered by new formulations and techniques which then led to medicinal chemistry to be possible[4].

The structure of the target is so crucial that we can even infer how the drug should bind by the possible interactions that could occur. A few examples of intermolecular bonding forces are hydrogen bonds and van der Waals interactions whose importance is paramount in structure based design –that, as the name suggests, is the strategy of design of a drug taking into account the structure and 3D arrangement of the biological target [5].In order for these studies to be possible, and to help evaluate the selection of possible drugs, computational methods are often employed. Molecular docking tests the potential orientations of a molecule in regard to other bio-

logically relevant molecules, ranking them in terms of their interactions to see if they would be likely to occur. The best results are those where the bonded compounds form a stable complex[6] .



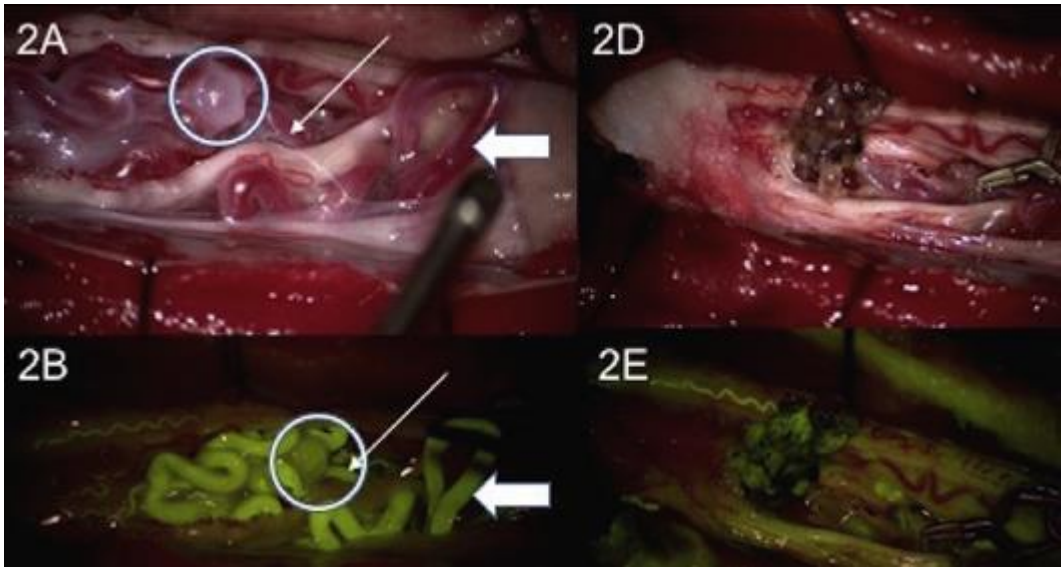
Scheme 42 – General representation of the docking process where a test is made to see if favorable interaction between the target (protein) and ligand would occur. After a screening with different ligand the best result is the one with the lower energy complex (molecular docking in the scheme). Scheme adapted from[7].

There are obviously some problems associated with this methodology, since docking is a theoretical exercise its main objective is to limit down the options which suggest unfavorable interactions. The main advantage of this methodology is that it gives a sense of direction to which parts of the molecules are "in contact", most importantly which interactions are more valuable for the decrease in energy of the complex that is created, thus making the bounding feasible. One of its several limitations is related to the fact that the biological targets, which are being tested are, most of the times, hindered from the direct addition of the drug, meaning that there is an intricate path that the drug must take to reach its objective. This path is usually quite difficult to undergo and the drug must possess specific characteristics to go through it. There is also the possibility that the drug experiences unwanted transformations or it reacts with some other targets which can lead to adverse effects. This can only be solved in *in vivo* studies which are difficult to test out in preliminary studies.

One possible approach to go around this situation is using *in vitro* tests in which a limited amount of cells is used to prevent the actions of other biotargets that would intervene in an *in vivo* assay. Even if a molecule is successfully screened in the docking experiment that does not mean it will be effective in the *in vitro* assay which seriously limits the method. We can safely say, that the extrapolation of the docking results to identify interactions on zones of molecular recognition has flaws associated with it thus it should be used with caution[8].

To this day, docking is considered a powerful and most essential tool for the discovery of new drugs as a theoretical exercise. The real question is if it is possible to map the actual binding between the drug and its biological target. This seems like a utopic preposition but there are some procedures which might allow us to reach this goal. The propriety that would shed light in this issue is fluorescence.

Fluorescence is a form of luminescence whereby a molecule emits after being excited by light of a shorter wavelength[9]. This description does not fully demonstrate the innate characteristics that make these tools so prevalent in analytic sciences. The method itself has a very large sensibility and sensitivity, for example, when compared to other absorption-based spectroscopies. Because most molecules do not have fluorophores, which consist on moieties which provide fluorescence to the system, it means that sensibility can be achieved. As sensitivity goes the fact that the emission is correlated to the amount of incident radiation means that it can be modulated to have higher values[10].



Scheme 43 – A Fluorescent compound (sodium fluorescein) being used as a corant for videoangiography. It is a non-evasive, reproducible technique to identify medical malformation in the colon[11].

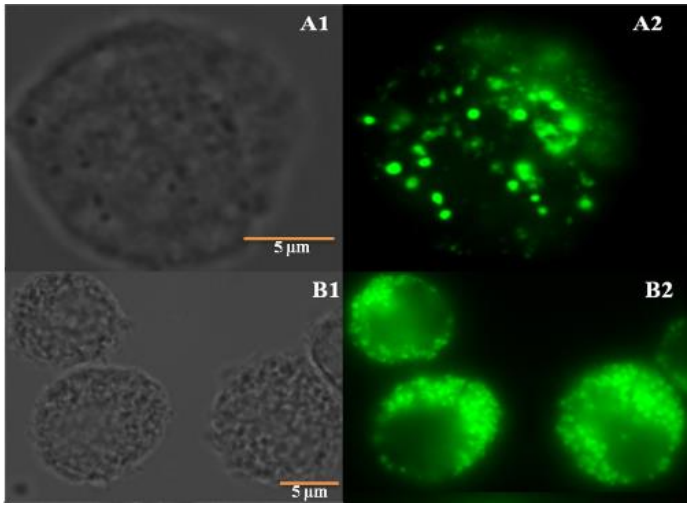
Even if a molecule is fluorescent it does not mean that all parts of the molecule share the same propriety, which can be explained by the process itself. The absorption from the pi system that leads to the consequent emission back to the fundamental state, is what prompts fluorescence. If a part of the molecule is not connected to that system, it means that it should not be visible by this technique.

At first sight, the information so far would lead to an erroneous affirmation: that one of the limitations of this technique is that it would only be useful to analyze interactions with fluorescent molecules. That is certainly not the case because fluorescence can be "added" to different compounds in a form of a probe. This term is nothing more than a molecule that is added to another without any fluorescence, naming in that case extrinsic fluorophore due to it not being intrinsic to the original compound.

It is most often used with microscopy because it allows for a visual comprehension of the phenomena. As a matter of fact, microscopes have become so powerful that it is possible to

distinguish parts of the same structural organelles within a cell, which is very important in to fully understand intramolecular processes and transformations [12].

In the last 60 years, there has been a significant development in both techniques that allowed for a constant evolution towards the final goal of molecular recognition (1-5nm). When that objective is finally achieved, it will substantially change our comprehension in molecular interaction due to the possibility of coupling it with the compatible fluorescent tagging [13].



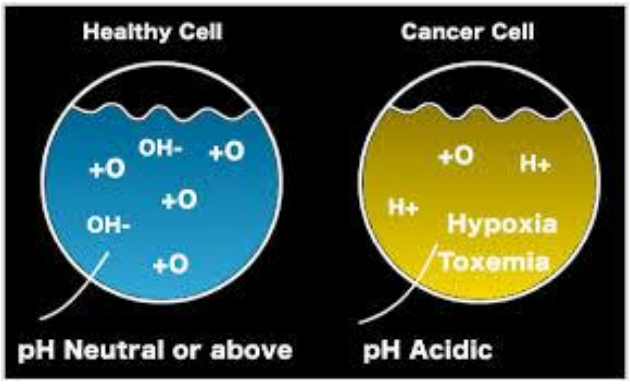
Scheme 44 – A scheme of fluorescence microscopy being used in a similar purpose to what is expected in this work. Scheme represents cells with endosomal markers[14].

When talking about this type of fluorophores, the two most important parameters are lifetime and quantum yield. The latter is the relative number of emitted protons versus the ones that were absorbed, which ultimately gives an indication of the brightness that can be achieved. The quantum yield is always less than the unity because of the losses that occur in the excited state. Graphically this can be easily seen by the stokes shift[15].

Also related to the excited state is lifetime, which logically indicates how much time the molecule spends in this high energy state prior to returning to the fundamental state. This factor is of upmost importance because it gives an idea of time available that the fluorophore has to interact or diffuse in its medium.

The interaction of the fluorescent molecule and its environment can dramatically affect its quantum yield and lifetime. When the fluorophore is attached to the target molecule, it usually ensues with a reducing effect on its previously mentioned parameters. Other environmental effect are, for example, solvent polarity and pH of the solution which are of major importance and can lead to interesting studies[16]. If one could modulate these parameters in order to achieve a maximum relative fluorescence at different pHs, one could also take advantage of this environmental sensitivity that can be very beneficial[17].

There is a very interesting subject that comes to mind when aqueous pH medium is broad up. One of the most important targets of study in pharmaceutical and medicinal fields is oncology [18]. It comes as no surprise that a huge amount of investment is made to uncover the processes and transformation that occur in the genesis of tumor formation. From all the known facts which are already confirmed and recognized, the one which is more important in the trail of thought of this work is that cancer cells usually have an acidic pH in contrast to other adjacent healthy cells [19].



Scheme 45 – Caricature about the parallelism between cancer cells and their acidity. Further explains about this topic in[20] .

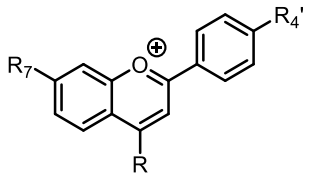
A great number of conditions must be fulfilled in order for a fluorescence probe to be considered viable. The most obvious one is related to biocompatibility because, even is a probe is attached to another molecule, if the linkage between them were to break, that meant that both molecules cannot be poisonous. Still in this point the probe must not be susceptible to degradation/transformations by biological agents that could alter its effects thus limiting its utility. As mentioned before in this introduction, the molecule must also be allowed to travel to its destination meaning that it must be able to pass through the supposed membrane channels[21]. Another condition that must be satisfied, which is inherently associated with the fluorescence parameters, is that the fluorescence must remain high at low concentrations, meaning it cannot be volatile during the whole process.

The proposal in which this project was built upon involves the use of compounds that supposedly exhibit all the characteristics that were mentioned before. The core of the biological probe we are suggesting is composed by the flavylium (2-phenyl-1-benzopyrylium) cation.

The presence of this moiety can be almost considered ubiquitous. One of the most pleasant things that we usually take for granted is color and that can be seen in many areas. Color can have many purposes which are intrinsically connected to today's society – for instance, the simple differentiation between men (blue) and women (pink) or the red from the green sign in traffic signals. Perhaps not as easily recognizable, is the "beautiful" aspect that can be associ-

ated by vibrant and effusive colors. Up to this day the most vivacious colors are attributed to nature. For centuries mankind tried to copy and use dyes to induce attractiveness to otherwise blend pieces. These components were only identified in the early 20th century and where named Anthocyanins[22] which in addition to color also are reported to add flavor[23].

The flavylium is nothing more than a simple chemical equivalent of anthocyanins that maintain some the characteristics that make these natural occurring pigments so interesting to study. One of them consists on the ability for the pH to modulate the color of the compound which is usually explained by to the presence of hydroxyl or other electron-donor groups. As a matter of fact, depending on the level of substitution on the flavylium which is being studied, it can have numerous forms at different pH.

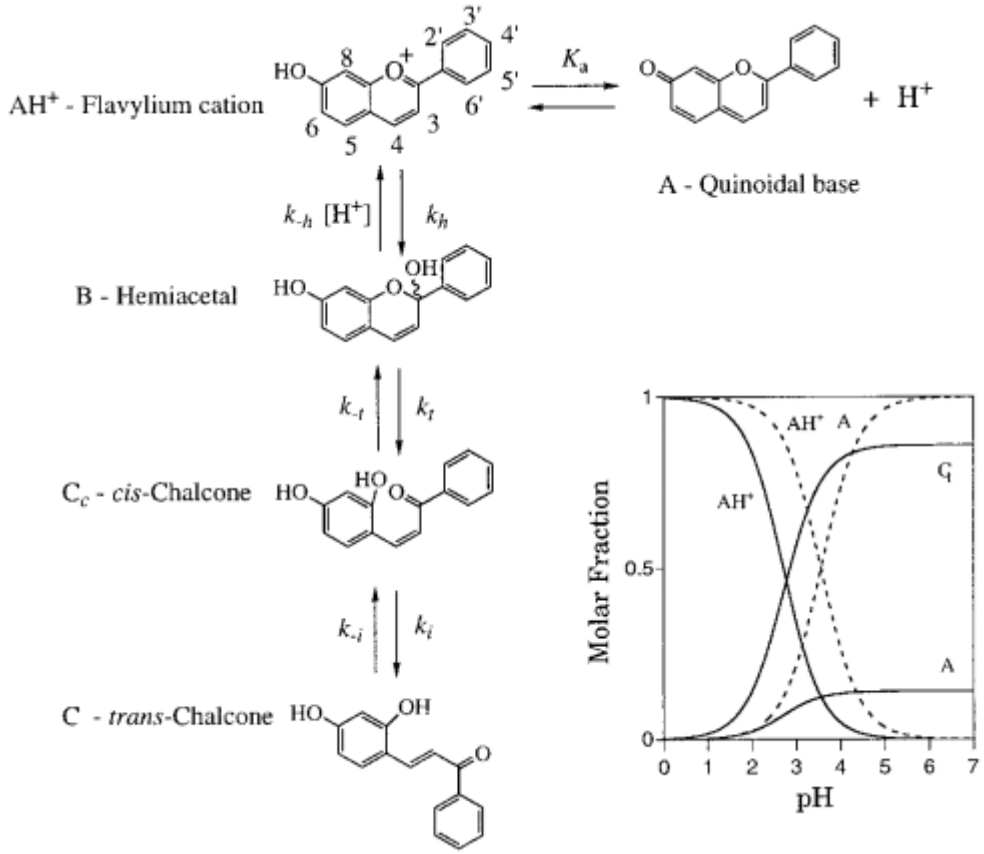


R= Substituion of the position C4 of the pyrylium ring

Scheme 46 – Generic representation of the flavylium salts used in this work. 4' substitution is on the phenyl moiety of the molecule. 7 substitution is on the benzopyrylium moiety. R consists on the substitution on the C4 on pyrylium ring.

Despite this being the case, the research on this molecule is in no way one-dimensional as can be proven by the range of application that have been developed after many years of investigation, some of which are : Studies on the color mechanisms [24], conceivement optical memory [25]and, for example , study on the biological effects of this type of compounds[26].

Despite the general disposition being rather simplistic there is associated with complex structural transformations[27]. To ease the comprehension of this fact a scheme with the different states in which the compound can exist. The example that was chosen was one of the simpler ones, the 7-hydroxyflavylium[28].



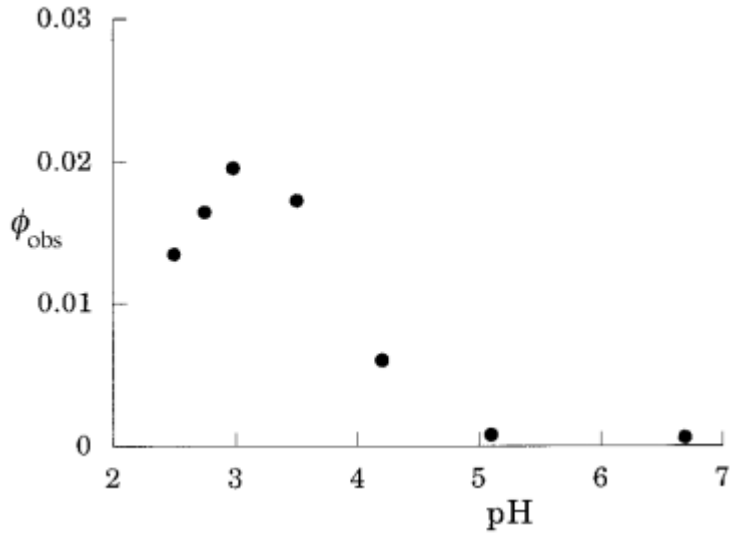
Scheme 47 – Schematic of the different species in which the 7-hydroxyflavylium system can exist[28].

From the analysis of the Scheme 5 different forms can be identified. The Flavylium cation can give rise to two of them by different processes. If a deprotonation occurs the quinoidal base is formed (A) leading to a neutral molecule that precipitates and for that reason no fluorescence is to be had in the same solvent. Same evidence can be seen if hydration of the flavylium cation takes place – this hemicetal (B) does not evidenciate any fluorescence properties. If a tautomerization in follows, the cis chalcone(Cc) is formed which can then be isomerized to the trans chalcone(Ct).

The study involved pH jumps in order to analyze the interconversion between the species. The increase pH of the medium leads to the formation of a precipitate which consists on the neutral quinoidal base.

From the graphic of the molar fraction of the species in relation to the pH of the medium, we can clearly see that at pH 3, the quantity in solution of the flavylium cation (AH^+) is substantially lower which is a problem due to it requiring really low pH values to exist in significant amounts. As discussed above, the acidity in cancer cells is evident, however , it does not have very low pH values – for that reason we must find a solution where the flavylium cation exists at higher pH's and even to neutral ones[29].

A very often employed answer for this problem involves the functionalization of the C4 of the pyrylium ring. It is known that the addition of aliphatic / aromatic groups increases the range of pH in which the compound is stable [30] owing to the inhibition of the formation of a possible hemiketal in the C4. If an aromatic substituent is used, this leads to the extension of conjugation, which in part, can stabilize the compound.



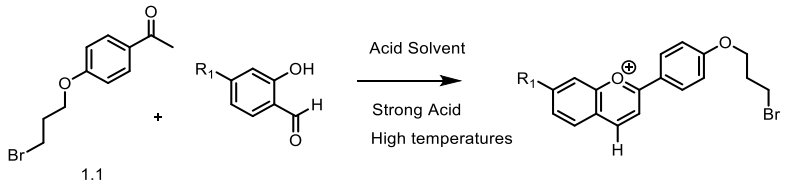
Scheme 48 – Quantum fluorescence calculation of 7-hydroxyflavylium at different pH's[28].

One very important fact to mention regarding this flavylium is that hydroxyl in the position 7 leads to a very limited fluorescence (quantum yield of 0.02 at pH 3)[28]. Firstly, as suggested by the different forms, the hydroxyl can leads to the formation of the quinoidal base. Secondly, the other reason is related to excited state proton transfer that undermines the fluorescence of the compound [31].

A possible substituent that would in theory tackle some of the problems presented so far would be the diethylamino group in the 7 position. One of the major differences between this and the hydroxyl group is that even if there is conjugation between the amino group and the rest of the molecule the flavylium continues to be a salt. This conjugation has also been shown to prevent the formation of the hemiketal on the position 2 even further (B) [32]. The last reason, nonetheless as important, is that it has been reported that the presence of the diethylamine group in the position 7 together with a donating group such as an alcoxide In the position 4' leads to a major increase in the fluorescence quantum yield which is crucial in this work [33]. This alcoxide substituent will be discussed further in this report.

For a large amount of time the main source of flavylium compounds was plant extracts which were complicated to isolate and expensive. The synthesis of flavylium salts was not only important to explore new compounds whose proprieties were unknown but also helped identify

the anthocyanins which were already isolated [34].The first synthetic approach gave name to a famous reaction whose importance in the field of flavylium formation is undisputed – Robinson annulation [35]. The reaction consisted on michael addition followed by an aldol condensation of acetophenone and salicylaldehyde which has proved to be very useful on the synthesis of natural products which have fused ring systems [36].

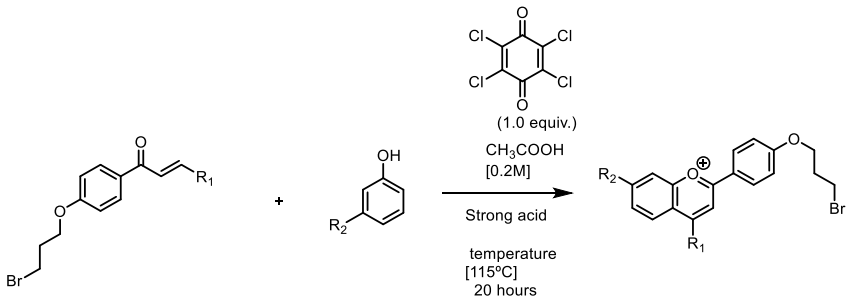


Scheme 49 - General representation of the flavylium synthesis using a salicylaldehyde derivative and the corresponding acetophenone (1.1).

The first attempt was made by using base to catalyze the reaction; however, certain flavylium salts have some problems if the medium is alkaline, as we have seen before. The acid catalysis was after this point, employed to generate the flavylium salt. The first example was using acetic acid and hydrogen chloride and it work quite handily[37]. The purification process remained similar to the one used today – crystallization using organic solvents.

The second most used procedure explored the already mentioned complex equilibrium that is inherent to the flavylium core. The utilization of a very strong acid with the appropriate phenol derivative and a chalcone can also lead to the desired flavylium by acid catalyzed condensation of both molecules. To the best of our knowledge this method was first used by Robinson in 1934[38] and it also used o-choranil as an oxidant that also increases the reaction rate.

In both methods there are advantages and disadvantages associated with them. The latter method using the chalcone can be useful to easily achieve a C4 functionalized flavylium salt. The possible limitation of this procedure is that some condensations are difficult to occur due to the nature of the substrates. The synthesis of some chalcones can also be problematic.



Scheme 50 – General representation of acid condensation to afford a flavylium salt substituted on a C4 substituted pyrylium ring.

The most used approach which, coincidently, was the first to be discovered, has a major benefit which is that, the acetophenone and salicylaldehyde derivatives are usually commercially available. The acid catalysis usually ensues without any major problem if the substrates are appropriated for the reaction. In both cases the acids which are used consist of protic strong acids such as sulfuric acid and hydrochloric acid. This last example can be used as a solution however, using it as an *in situ* gas is also common [39].

In order to achieve the functionalization on the C4 that was previously discussed, the most common method was popularized by Katrizky and consists on the umpolung reaction using benzotriazole [40].After the addition at C4, the chemical proprieties of the benzotriazole allows for the *ipso* proton to became acidic. After this step the proton is removed using a strong base, usually alkyl lithium, and an electrophilic reagent is then added for substitution to occur. When the reaction is quenched and extracted, the benzotrizole is removed and the electrophilic reagent stays in the C4.

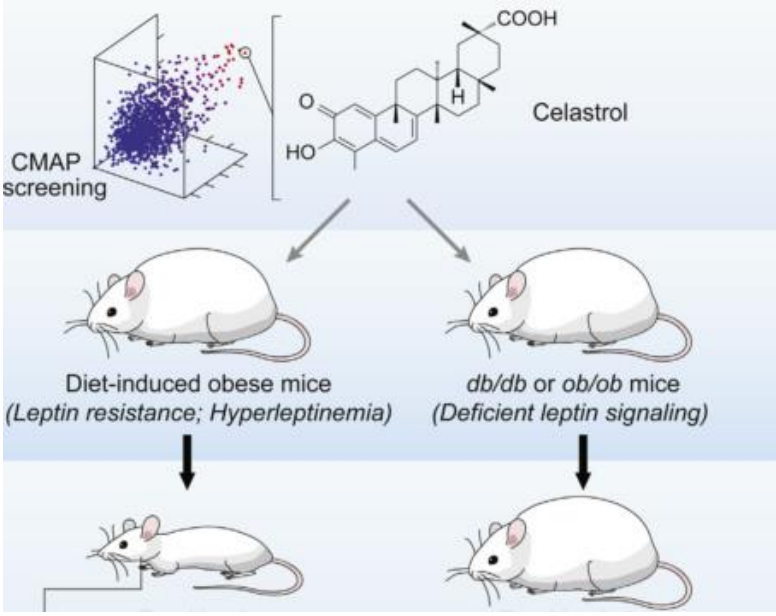
Now that we disclosed and analyzed our preposition of a fluorescent core that would allow us to identify by fluorescence microscopic spectroscopy we need to decide on a possible drug that would allow us to investigate certain biological targets. One important fact to ascertain before the drug is revealed is that there must be a linkage between both of them. This linker must meet some requirements, essential for the successfulness of the probe itself. The ideal linker must be chemically inert and easy to connect to both molecules. The 4' position of the flavylium should be an appropriate place to have one part of the linker due to the previously discussed need to have a donating group at that position to increase the fluorescent quantum yield. The size of the linker is a controversial topic due to their existing arguments, which corroborate opposing sizes. A balance must be reached in both sizes of the spectrum.

If a small linker is employed there is a smaller surface area in the drug that could impedewith its possible interaction with the biological target. If the linker is too small there could even exist conjugation with the drug that could lead loss of fluorescence or even modification in the drugs composition that would prove to be problematic. The large linkers would give a high level of flexibility to the probe which could potentiate intramolecular interactions between the fluophore and the drug[41].The conformational freedom that would be attributed in this case would also difficult the interactions with the active center [42]. We opted for an intermediate linker that consisted on an aliphatic 3 alkylic chain.

Now that a strategy has been defined regarding the size of the linker that connects the probe and the promising pharmaceutical compound, the only way to verify if the chosen size is optimal is to empirically prove it.

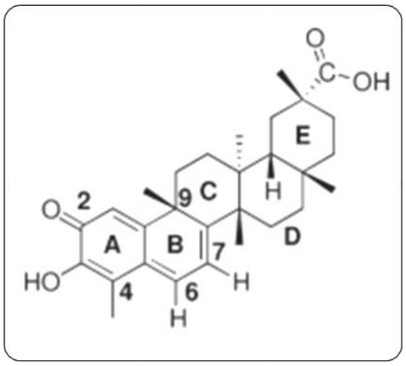
The best case scenario as far as the drug is concerned would be a relatively known biological compound whose activity was very high however its interactions were not fully understood by now, it also had to have a moiety that could be explored to connect the linker and the fluorophore. As a matter of fact we were able to experiment with a compound which shared such characteristics.

Celasterol is the most biologically interesting compound that was isolated from the extraction of *Tripterygium wilfordii* which has been used for centuries in chinese medicine. It is important to mention that the use of this medicine has, as it is usual in these cases, some secondary effects mainly gastrointestinal. The therapeutic results have been consistently interesting and are related to many clinical areas such as inflammatory diseases [43]and obesity treatments[44].



Scheme 51 – Adapted scheme of the effect of Celastrol in the treatment against Obesity - diminishing the appetite. Celastrol was found by CMAP(Connectivity map) screening to be the best candidate for this function.

Other major effect that celastrol exhibits is its antitumoral activities which, for reasons we already discussed when mentioning the fluophore, is really interesting to us. These effects were demonstrated in *in vivo* tests with cancer models in which suppression of the tumors growth, metathesis and progression occurs [45]. It is well documented that metastases are responsible for >90% of cancer related deaths[46] which celastrol is documented to inhibit preventing the anti- invasive activity[47].



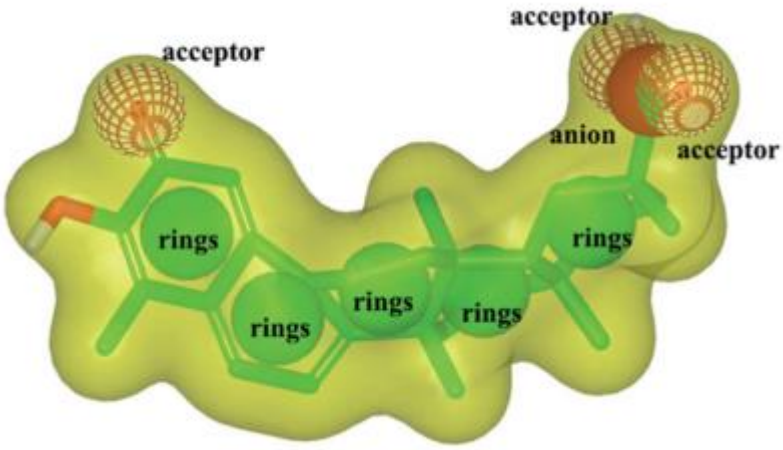
Scheme 52 – Celastrol structure representation and its division into 5 different ring (from A to E).

Although the exact mechanism is not known, by using derivatives of celastrol, an idea of the importance of the moieties in question can be acquired. This method has been used extensively and yielded various derivatives which also exhibit very interesting therapeutic activities, some of which we will soon discuss.

By replacing selected parts of the molecule in each alternative compound a number of important relations could be made. Perhaps one the most vital for the continuation of our work was that, in ring E, if the acid group was transformed into a methylester the compound remained most of its biological activity [48], which opened a pathway to the linker to be connected. As a matter of fact some of the problems associated with celastrol are also diminished with this deretivization. The low solubility and high toxicity are known to become less relevant when the ester is present [49]. From this point on we knew that the linker had to able to do an ester bond between the molecules.

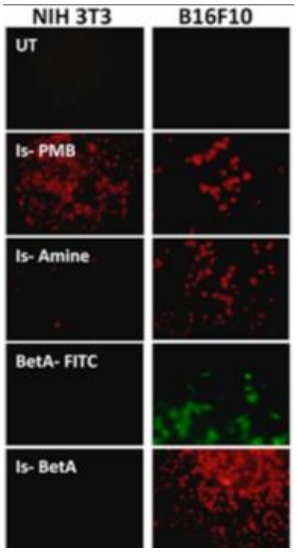
The A-B ring compose the most exotic part of compound, the quinone methide, which has been shown to have major biological significance it terms of its role in celastrol's effects [50]. This moiety is described as a hydroxyl group adjacent to a carbonyl in conjugation over two 6 membered rings (A-B). In regards to the rest the compound the specific structural environments and sterics must play an essential role in its reactivity and stability [50].

As we mentioned before in this introduction, computational methods are really important tools to access more information to corroborate with experimental data that has been already acquired. A virtual screening of celastrol and its derivatives also showed possible intermolecular interactions that could occur with the selected biological target. Some of which being that the hydroxyl group on ring A can provides polar interaction and the carbonyl group on the acid group / ester in ring E hydrogen bond accepting proprieties [51].



Scheme 53 – ROCS model representation [51] with possible Celastrol interactions with other potential compounds.

One very interesting case study that shared the general purpose of this involves betulinic acid with a fluorescent probe composed by bis-arylidene oxindole. Just like celastrol betulinic acid is a triterpenoid which shares the most of its therapeutic proprieties. It is important to mention this paper because, it proved that the general concept of our work is possible – to selectively identify and destroy cancer cells [52].



Scheme 54 - Fluorescence microscopy results using different compounds in different intracellular mediums – NIH 3T3 (normal cells), B16F10 (Cancer cells). Is-PMB (Isatin with PMB group), Is – amine(Isatin with an 3 alkylic amine linker), BetA- FITC (betulinic acid with Fluorescein isothiocyanide), Is- BetA (Isatin coupled with betulinic acid).

It is possible to visually recognize if there is an uptake of the pharmaceutical compounds to cancerigenous cells. In this example we can see that the selectivity of the different compounds to the different medium is not the best because non active molecules such as the

fluorescent probe (isatin) are being consumed during the test. This work still had a very interesting and promising proposition which we tried to take advantage of.

This work's proposal tried to overcome some of the difficulties that were encountered in this early example – being that our probe consists on a flavylium moeity its solubility is supposed to be higher in aqueous media. Other point to be noted is that the probe itself showed toxicity issues which should not be encountered in a natural occorent compound such as a flavylium salt.

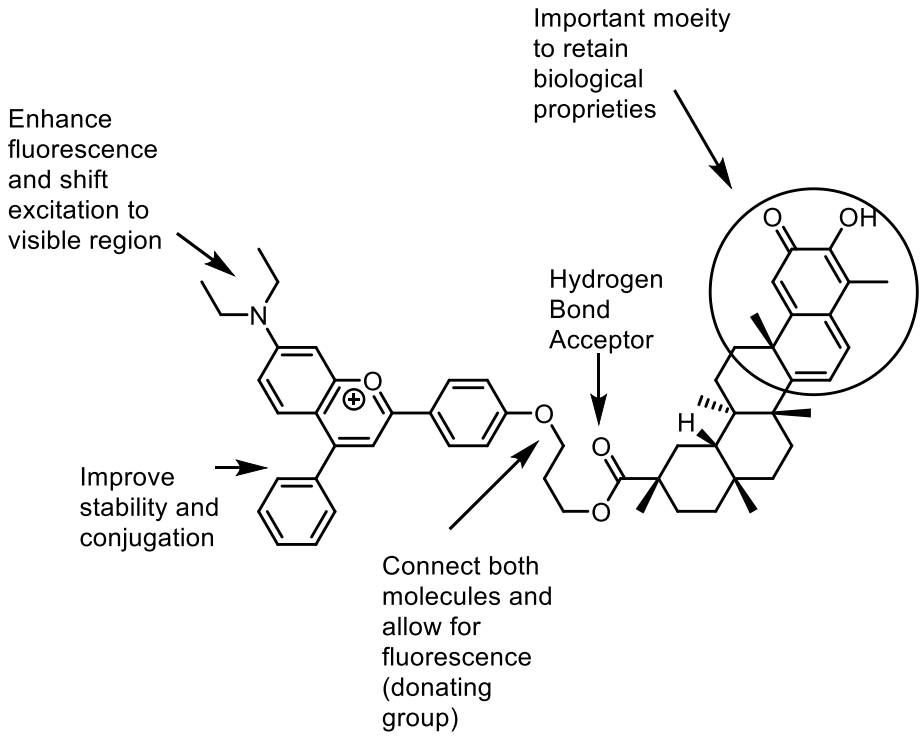
Yet another stimulating study involved the functionalization of the position 6 with indole derivatives that afforded a significant activity increase in the *in vitro* tests, that also suggests that the Michael acceptor moiety is as important in the anti tummoral effect[53].If these powerful derivatives where to be discovered that would consist on a wondrous development in cancer treatments. Moreover if these compound where to be coupled with a flavylium fluorescent probe that would allow for a biological mechanistic understanding of the entire process.

II-Discussion

II.1-Synthesis of molecular probes using flavylium core

Initial contemplations

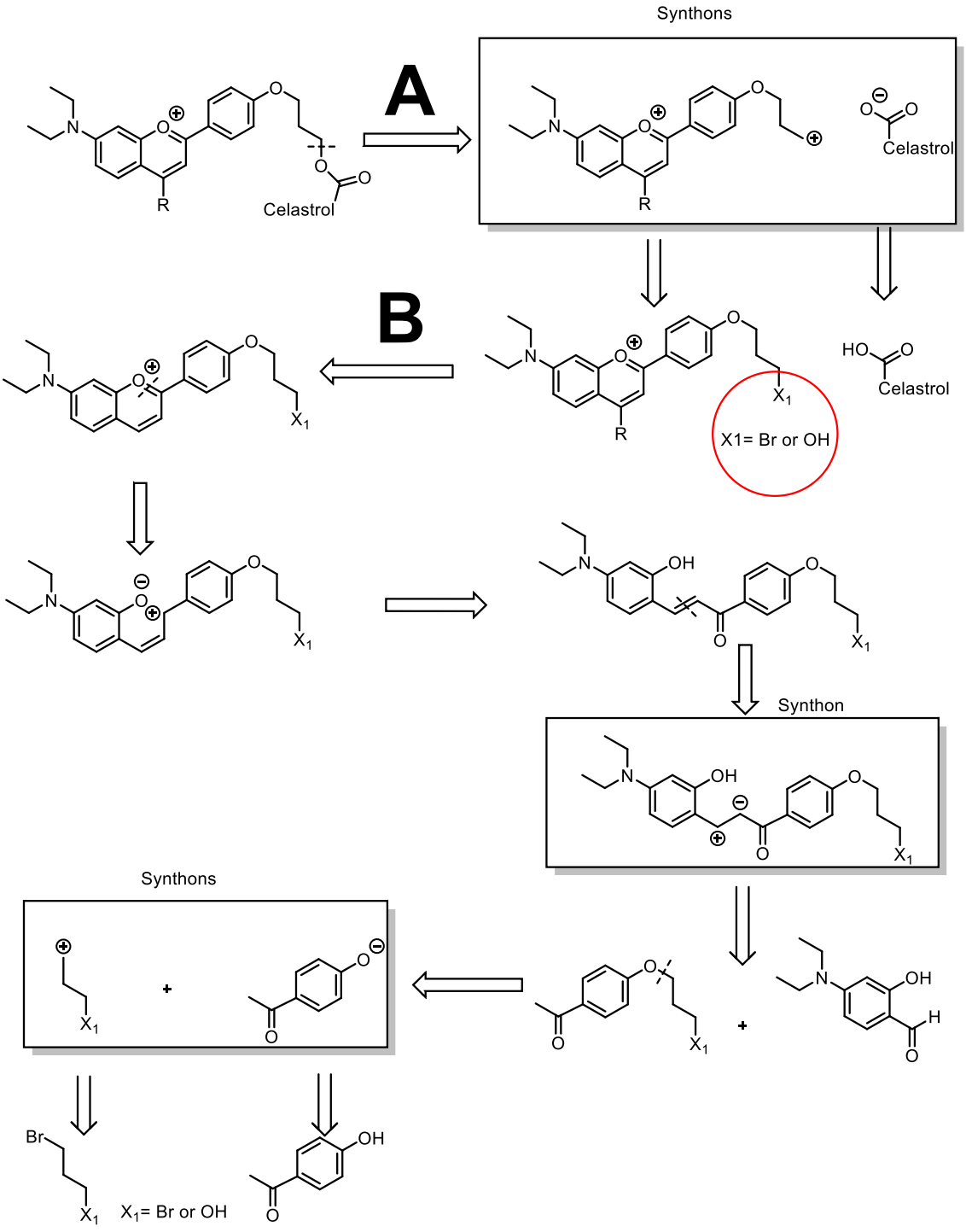
We would like to reinstate a few points that are inherently related to the work we have proposed to do: the general outlines are related to the synthesis of a molecular probe using a flavylium core that, in theory, confers fluorescence to a pharmaceutically active compound. The newly acquired properties of the molecule would help elucidate the mechanism of action of the drug in the cellular environment using the fluorescence to guide the process. The synthesis of the flavylium would include a molecular linker to serve as a connection to the drug and a diethyl amino group in the 7 position to enhance its fluorescent characteristics and allow for excitation in the visible region.



Scheme 55 – Schematic of the final product with detailed information on the regions which are important to be present in the finalized product.

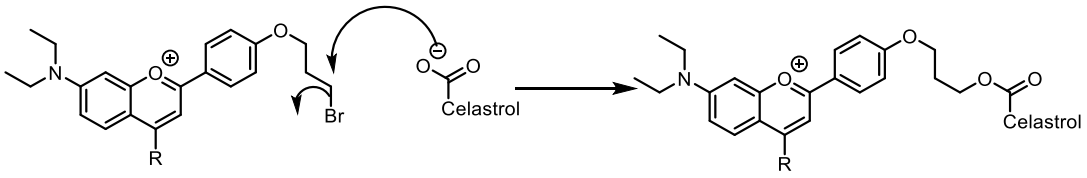
A very important tool that must not be overlooked when talking about synthetic alternatives is retrosynthesis. This process whose practice in chemistry is of the upmost importance was only branded in the past few decades [54].However, it has been used, perhaps unconsciously, since synthetic chemistry came to be. It consists on a logical process that backtracks the approach to the target molecule, meaning that it uses bond breaking to reach theoretical reagents that would undertake the transformation that was previously cleaved.

Its applicability is immeasurable especially in the realm of total synthesis where complex molecules with intricate functional groups require, most of the times, ingenious ways to synthesize them. In order to assist us in our work we used this important process to outline different strategies that would help us reach the desired compound (scheme 15).



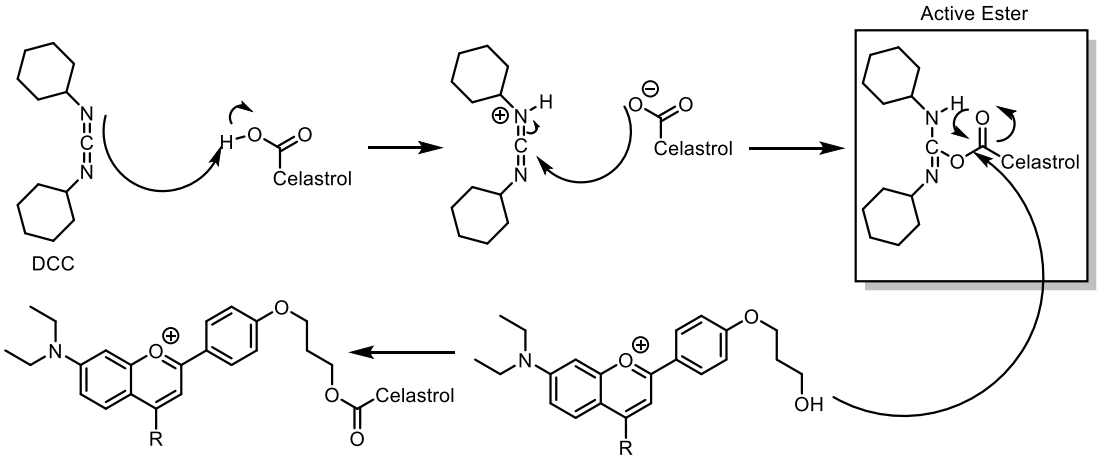
Scheme 56 – First retrosynthetic approach to the formation of the celastrol coupled to the desired flavylium salt.

There are two retrosynthetic disconnections that deserve special attention (Scheme 16). The first one is related to the coupling between the two final molecules, the flavylium salt and the pharmaceutical compound, celastrol. As represented in the scheme 16, the bond between both molecules can be made by several different ways being the most common the ones we are going to discuss ahead. The first one and perhaps the simplest is by generating a carboxylate on the acid moiety of the celastrol. When the anion is formed, it can undergo SN2 reaction of the terminal chain halogenated (X_1 = Br in scheme 16)3 carbon alkylic dimer.



Scheme 57 – Reaction with the carboxylate salt of celastrol and a flavylium salt with a halogenated carbon linker.

The second route is by activation of the carboxylic acid to perform an esterification. This transformation is usually promoted by coupling reagents such as carbodiimide [55]. The reaction itself can be catalyzed by other compounds such as DMAP to enhance the rate of the transformation.

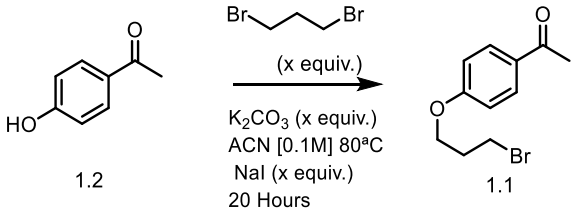


Scheme 58 – Proposed mechanism for the coupling between the flavylium salt with an alcohol linker and celastrol. DCC was used as an example of carbodiimide[55].

The other disconnection pathway that should be discussed is related to the insertion of a group to the C4 position of the flavylium whose importance has been mentioned in the introduction. The most studied method to achieve this transformation is by means of an umpolung reaction using benzotriazole. After the addition of the benzotriazole under basic conditions, the proton on the C4 position is susceptible to being removed. This fact makes for the possibility of a substitution of an electrophile to happen.

Preliminar approach to the flavylium core

As it was noted during the introduction the flavylium core is very sensible to alkaline conditions, it was decided then that the alkylic chain-linker was supposed to be attached before the annulation that leads to the formation of the pyrylium ring took place. The logical path meant that alkylic chain was to be attached to 4-hydroxyl acetophenone, before the condensation (Scheme 19).



Scheme 59 – Substitution of 1,3-dibromo propane by 4'-hydroxy Acetophenone in K_2CO_3 and NaI at 80 ºC.

Table 16 –Results relative to the reaction in scheme 19.[a]Starting material was recovered. Work up consisted on the washing with brine (2x). Purification using a flash chromatography hexane / ethyl Acetate(9:1) , all reaction were conducted in a 5mmol scale The solvent was acetonitrile [0.1M] and the reaction time was 20 hours in all cases. Reaction temperature of 80°C.

Entry	K_2CO_3 (equiv.)	Catalyst NaI (equiv.)	1,3-dibromopropane (equiv.)	Yield (%) 1.1
1	5	-	3	47[a]
2	5	0.1	3	64[a]
3	8	0.1	3	55
4	5	0.15	5	72
5	5	0.25	5	80
6	5	0.1	5	74
7	4.5	0.1	5.5	77
8	5	0.4	5	78
9	5	0.5	5	85
10	5	0.75	5	78
11	5	1	5	80
12	5	1.5	5	82

The first synthetic approach began with the reaction of a 4'-hydroxyacetophenone with 1, 3-dibromopropane in a basic medium (Scheme 19). Similar reported reactions were conducted with brominated carbon linkers and KI as a catalyst [56].Due to the fact that the bromine atom is a good leaving group the catalyst was not added in the first attempts. When NaI was used the

yield improved significantly and its optimum amount was 0.5 equiv. (ranged between 0.10 to 1.50 equiv.; Table 1).

In this first transformation we can clearly see the effect of the catalyst on the reaction's outcome. When the sodium iodide was not used the reaction occurred rather slowly even at high temperatures (entry 1). In this case starting material was recovered in substantial amounts suggesting that a catalyst would be necessary for the reaction to occur. It is important to note that no elimination product was isolated in this case.

The only side product that was found to occur in this reaction was the elimination product on the 3 carbon linker. The reason behind its formation is the alkaline medium and the high temperature reaction in which the reaction takes place favoring the elimination reaction. Nevertheless, the purification process that was developed did not easily separate both products due to similar Rf's. Other flash chromatography solvent systems were used however no improvement was seen in comparison to the original conditions.

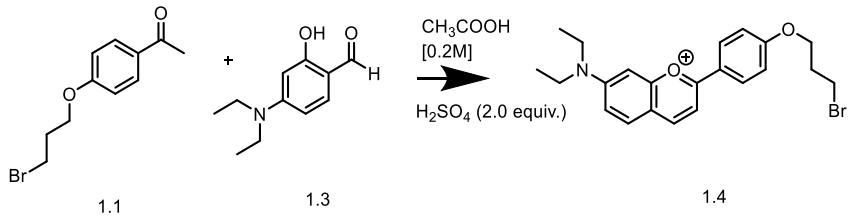
The amount of base that was used did not shown to have a major role in the reaction effectiveness mostly because of solubility issues (entry 3) in organic solvents, thus we decided to grind the potassium carbonate pellets, however, no significant change was observed.

The amount to electrophilic reagent was also analyzed, no change in the overall yield of the reaction was observed (entry 4).

A correlation can be made with the amount of isolated product and the catalyst quantity that was used. The best result was found when an intermediate concentration of sodium iodide was employed (entry 9). One possible explanation for this outcome is directly related to the results when higher quantities of catalyst were added to the reaction's medium (entries 10-12). In these latter cases the elimination product was showed to increase most likely because the exchange of the bromine atom by iodine that leads to its elimination. We can safely assume that an intermediate quantity of sodium iodine was the best condition for this reaction.

The only side product that was found in this reaction was the elimination product on the 3 carbon alkylic linker, due to the alkaline medium and the high temperature reaction in which the reaction takes place.

Having compound 3 bromo-4-propoxyacetophenone (1.1) in hands, the next step consisted on building up the flavylium skeleton by one of the first methods to generate that moiety (Scheme 5).



Scheme 60 - Condensation of 3 bromo-4-propoxyacetophenone (1.1) and 4-diethylaminosalicylaldehyde (1.3) [1.0 equiv.] in acetic acid [0.2M] and sulfuric acid (2.0 equiv.). Reaction was performed in a 2mmol scale.

The formation of the flavylium involved an acidic Robinson Annulation with H_2SO_4 in acetic acid. After the slow addition of sulfuric acid the color of the solution changed from uncolored to bright red. The TLC demonstrated a very fluorescent spot in the application point meaning that the reaction was successful.

A solubility test was performed with several organic solvents in order to identify the best for product precipitation in larger quantities. Much like in every attempt when the a flavylium cation was synthesized in this work the selection of organic solvents were: Chloroform; Ethyl Acetate; Diethyl ether ;THF ; Methanol; Dichloromethane; Hexane.

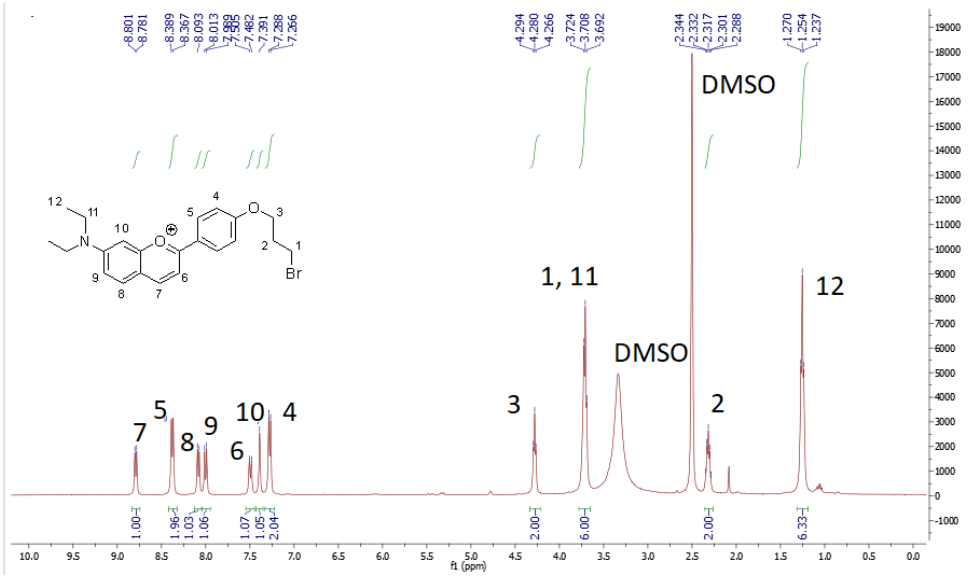
The best results as far as the precipitation is concerned were obtained with ethyl acetate and diethyl ether with the latter showing a significant improvement. The solution was passed through a fritted funnel and it was not retained in it, most likely due to the size of the particles. The precipitate was considered an oil and the sulfuric acid could not be removed in any way. This fact was quite problematic due to a number of reasons: first it was not possible to quantify the reaction meaning that an idea of the yield was unconceivable; secondly because the oil was very acidic it meant that the crude could not be used in a sensitive follow up reaction and third, it was quite difficult to successfully remove the contaminants of the reaction because there was no appropriate purification procedure.

Several attempts to purify the oil were attempted. Extraction was the first option, however problems arose from it. The oil and its contaminants remained in both the aqueous and organic phase at different pHs and organic solvents, this meant that most of the product was lost in each extraction. As a matter of fact the first purification of the flavylium by Robinson involved the extraction with isoamylic alcohol[37] and even this was unfruitful in this case.

Because we knew that the compound did precipitate we experimented on decantation with the hopes that the compound would be purified in that way. What we observed was that a lot of compound was lost during the procedure. The next logic step was to use other purification techniques like column chromatography. The purification of flavylium salts by them is scarce[57],

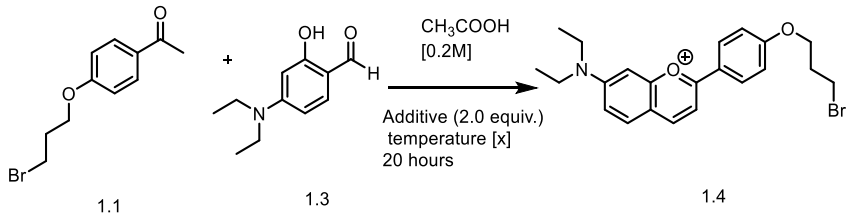
because of the sensitivity of the substrate itself. Various types of stationary phase and solvent systems were tested and the results were similar and disheartening. If the flavylium did not decompose during the column it would remain stuck in the stationary phase and decompose when flushed out.

After setting up the same reaction on a larger scale (10 mmol) and washing the product we were successful on cleanly characterizing the flavylium product. After all the procedure, only 20 mg of a red oil were obtained with the following ^{1}HNMR spectra(Scheme 21).



Scheme 61 – ^{1}HNMR spectra of the 7-diethylamino-4'-((3 bromo)propoxy)flavylium(1.4) in DMSO-d6 with the corresponding structure assigned using 2DNMR.

In order to try to solve one of the issues of the previous method we substituted the sulfuric acid with other strong acids with lower boiling point in order to be able to completely dry the crude.

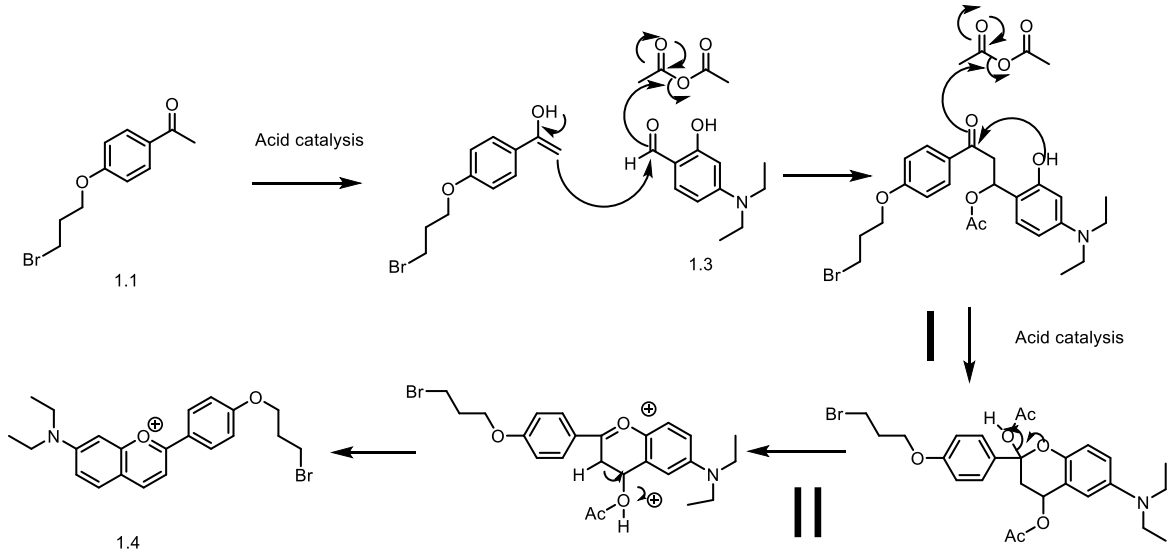


Scheme 62 - Condensation of 3 bromo-4-propoxy acetophenone(1.1) and 4-diethylaminosalicylaldehyde (1.3; 1.0 equiv.) in acetic acid[0.2M] and additive (2.0equiv.) for 20 hours.

Table 17 – All reaction were conducted in a 3mmol scale of 3 bromo-4-propoxyacetophenone with 1equiv. of 4-diethylaminosalicylaldehyde. Acetic acid was used as a solvent [1M] and the reaction was monitored for 24 hours. Reaction was performed in a 2mmol scale.

Entry	Strong Acid	Additive	Temperature [ºC]	Observations
1	HBF_4	Acetic Anhydride [1M]	70	Changed color faster than the others
2	HBF_4	-	80	-
3	HCl (g)	-	50	-
4	H_2SO_4	-	100	Difficulty in removing the solvent

The first choice was HBF_4 that was selected mostly because of numerous examples in literature [40]. The same acidic solvent was maintained in order help catalyze the reaction and to allow the comparison with the sulfuric acid reaction. Two variants of this reaction were attempted with similar results. Experimentally the major difference was that when acetic anhydride was added the solution only changed color (entry 1). The role of the acetic anhydride has not been explained, to the best of our knowledge; however it seems to be crucial for the reaction. A possible explanation could pass by the acylation of the alcohol to facilitate elimination (Scheme 23; Steps I and II) speeding up the transformation.



Scheme 63 – Proposed mechanism for the condensation 3 bromo-4-propoxyacetophenone (1.1) and 4-diethylaminosalicylaldehyde (1.3) in acid conditions using acetic anhydride.

Without the use of acetic anhydride the solution only turned red when the acetic acid was already in ebullition (entry 2).

We repeated the same workup and found an identical situation to what was previously presented. After some decantation using diethyl ether and the evaporation of both acids using azeotropic mixtures [58]a black solid appeared. After characterization it was found that the flavylium had decomposed and could not be used further. Explanations for this fact could be that the high temperatures and low pressures used led to the degradation of the product. Another reasoning behind would be that this particular flavylium requires an acidic amount of solvent in its core to remain stable[59].

Now that we knew that full evaporation was not possible we focused on the particle size of the product. We theorized, after noticing that a suspension was always formed, that centrifugation could help in removing the supernatant form the rest. The decantation had to done rather quickly, after the solution stayed in the freezer after some time. Using this procedure we were able to identify the flavylium from ^{1}HNMR spectroscopy with large expected amounts of solvents still in the crude.

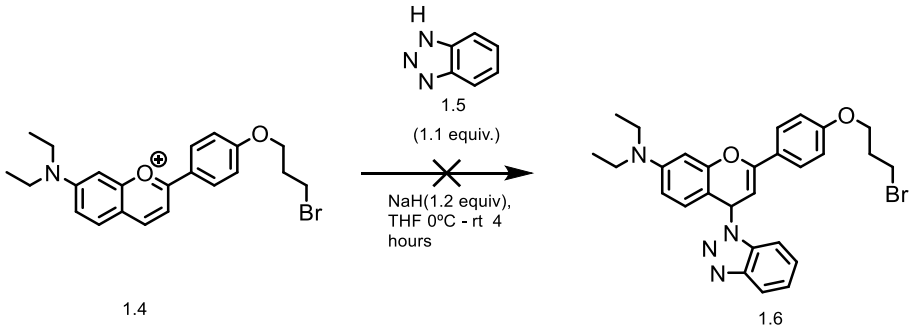
In the synthesis of salts one very important factor to take into account is the counter ion. It is known that there are some characteristics that can be modulated by the type of anion that is used. BF4- is a non-coordinating anion that is supposedly inert. In order to test if the type of anion would enhance or even change the precipitation process, efforts were done to change it. The counter anion we chose was Cl^- – that, unlike $BF4^-$, is a hard nucleophile that perhaps could improve the precipitation. Because an exchange column was out of question we first attempted the exchange by refluxing the flavylium with a solution of hydrochloric acid. After that procedure we could not distinguish from the previous flavylium.

To prove that the counter anion did not have a significant role in the precipitation of the product we repeated the condensation reaction with gaseous HCl (entry 4) formed *in situ.* This was also a very popular way to generate the flavylium[60] , this time with the Cl^- counter anion. The purification procedure was maintained and no obvious difference from the original method was noticed.

At this point we decided to continue with our planned synthesis and proceeded to the umpolung reaction using benzotriazole (Scheme 25).

The benzotriazole addition to the C4 of the pyrylium ring was not successful. In this reaction numerous variations were experimented with a focus on the order of reagents addition.

Some difficulties were expected because of the nature of the reaction and the vestigial solvent left in the flavylium salt. Another important fact that is important to discuss is that, to the best of our knowledge, this was the first addition of benzotriazole to a flavylium salt that had two possible locations for addition / substitution to occur.



Scheme 64 –Addition of benzotriazole to the 4 position of pyrylium ring of the 7-diethylamino-4'-((3 bromo)propoxy)flavylium(1.4) with 1.1 equiv. of benzotriazole (1.5) and 1.2equiv. of NaH in THF at 0 to rt for 4 hours.

Table 18 – All reaction were conducted in a 2mmol scale using 7-diethylamino-4'-((3 bromo)propoxy)flavylium (1.4) and 1.1equiv. of benzotriazole (1.5) with 1.1 equiv. of NaH. The reaction was monitored for 4 to 7 hours and then quenched. The starting material was not recoverable because of degradation.

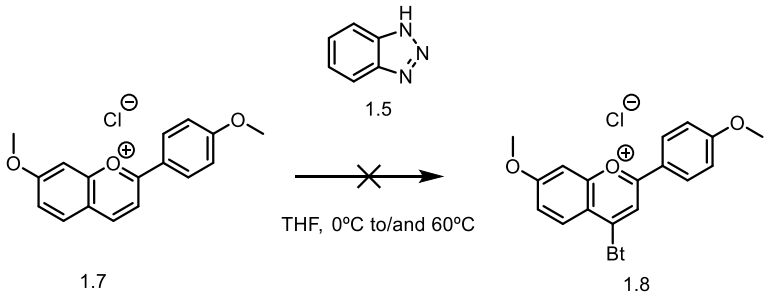
Entry	Order of addition	Temperature [ºC]	Observations
1	Normal	0	No reaction
2	Normal	rt	No reaction
3	Normal	60	Decomposition
4	Inverse	0	No reaction
5	Inverse	rt	No reaction
6	Inverse	60	Decomposition

A series of different reaction conditions were studied and analyzed. In regards to the order of addition when the solution of benzotriazole salt was added to the flavylium. The reaction was monitored by TLC using the consumption of benzotriazole as control. An experimental detail that we always verified was the pH of the solution which, always showed to be acidic, meaning that the residual acid neutralized all the hydride. If a very large excess of base was employed it most likely would lead to the opening of the flavylium rendering the reaction useless.

In literature the benzotriazole anion is always used in this reaction. We thought that the reason behind it was to speed up the process. Because the flavylium we were using had quite peculiar characteristics that prevented the full purification we decided to use a simpler flavylium salt to verify if the reaction was possible without any base to catalyze it.

Initial studies on the addition to the C4 position of the pyrylium ring

In this preliminary attempt we could clearly see that no reaction occurred in the absence of sodium hydride, even with the increase in temperature. In order to prove this point the reaction with sodium hydride was experimented and the reaction did not yield the expected product.



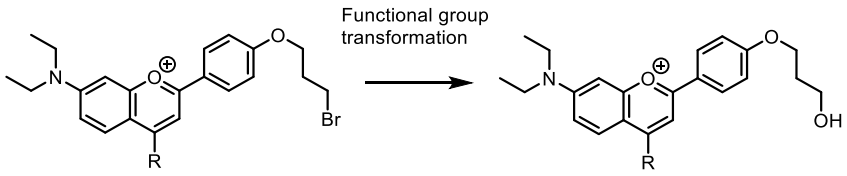
Scheme 65 - Addition of benzotriazole (1.5; 1.1equiv.) to the C4 of the pyrylium ring of the 4'metoxy-7-metoxyflavyliumchloride (1.7). Addition was done at 0ºC and was heated to 70ºC, yet no reaction occurred.

The reaction temperature also played an important role in the reaction outcome; the kinetic, low temperature product, was the addition to the C4 position, however, this can only be confirmed by experimental data. Some experiments were conducted in a range between 0ºC and 60ºC.

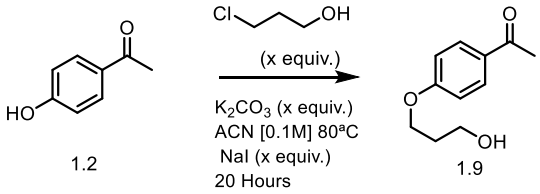
Even though no product of this reaction was isolated and characterized the fact that there were two locations for the benzotriazole to attack meant that pursuing this method was very time consuming with a high possibility of failure. To elucidate this fact we found that the studies on regioselectivity regarding benzotriazole additions are few [61]and almost exclusively related only the addition to the *ortho* versus *para* position(C2v C4)[62].

Alternate linker possibility – Propoxy functionalization.

A new synthetic approach was formulated to solve the problem of selectivity between the position 4 of the pyrylium ring and the bromine in the 3-carbon alkylic chain. The proposed solution was the substitution of the bromine atom to a hydroxy group. This change would render the an inert position in respect to the nucleophillic attack of the benzotriazole anion. This group could be later converted to an halogenated atom[63] in order to be attacked by the carboxylic acid of the celastrol molecule. Yet another possibility involved the direct esterification between the acid and the alcohol.



Scheme 66 – Proposed transformation to afford the hydroxyl linker to facilitate final esterification with Celastrol.



Scheme 67 - Substitution of 3 chloro-1-propanol (1.8; x equiv.) by 4'-hydroxyacetophenone (1.2) in K_2CO_3 and NaI at 80ºC. Work up consisted on washing with brine (2x). Purification using a flash chromatography hexane / Ethyl Acetate (5/1).

The first step of introduction of the linker was very similar in both pathways (Scheme 28) being the only difference the use of 3-Chloro-1-propanol instead of 1, 3-dibromopropane. The yield of this reaction was quite high if the sodium iodide catalyst is used.

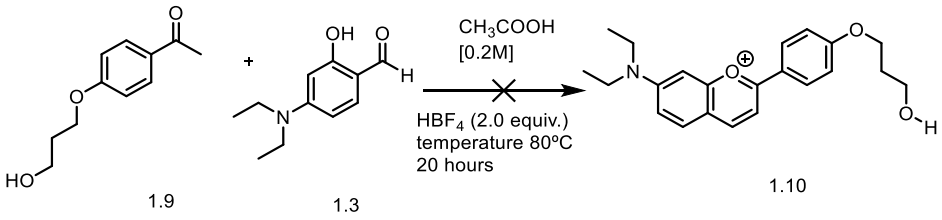
Table 19 - All reaction were conducted in a 5mmol scale of 4'-hydroxy Acetophenone. The solvent was acetronitrile [0.1M] and the reaction time was 20 hours in all cases. Reaction temperature of 80ºC.

Entry	K_2CO_3 (equiv.)	Catalyst NaI (equiv.)	3 Chloro-1-propanol (equiv.)	Yield (%) (1.8)
1	5	-	3	50
2	5	0.1	3	62
3	5	0.2	5	67
4	5	0.5	5	72
5	5	0.7	5	78
6	5	1	5	82

Logically some comparisons can be made in regards with the substitution reaction using 1,3 dibromopropane to introduce a different carbon linker. Perhaps the major difference is that the amount of unsatured product that comes from the elimination of the leaving group from the linker is inexistent in contrast to the latter case. This fact is very important because it facilitates the purification even further. One can also comment of the fact that higher quantities of catalyst lead to higher amounts of product.

In general this reaction was much cleaner than its bromine counterpart which is to be expected.

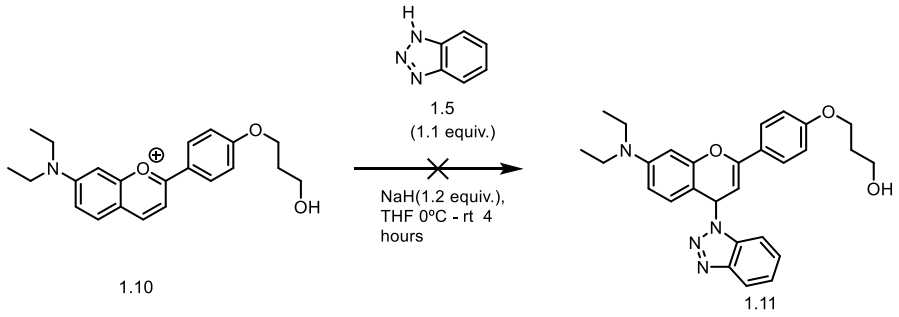
The following step involved once again an acid Robinson annulation which occurred with HBF_4 and Glacial Acetic acid under reflux. The problems with the work up persisted and the product remained an impure oil. As a matter of fact the ^{1}HNMR spectra were more difficult to analyze most likely due to the exchange between the residual solvent and the free alcohol in the product.



Scheme 68 - Condensation of 3 hydroxy-4-propoxyacetophenone (1.9) and 4-diethylaminosalicylaldehyde (1.3; 1.0 equiv.) in acetic acid [0.2M] and HBF_4 (2.0equiv.) for 20 hours at 80ºC. Reaction was performed in a 2mmol scale.

Another comment that can be made is that the evaporation of the majority of the solvent, resulted, in some cases, in the exchange of color from red to purple which indicates the degradation of the compound. These results suggest that the propoxy flavylium (1.10) was less stable and needed careful attention when evaporating its solvent.

Like what happened in the previous synthetic path the benzotriazole addition was not successful. The increase in temperature and order of addition did not show any meaningful influence on the outcome of the reaction. One additional interaction to consider would be that the sodium hydride has enough strength to also remove the proton form the linker's alcohol which then could lead to side reactions.

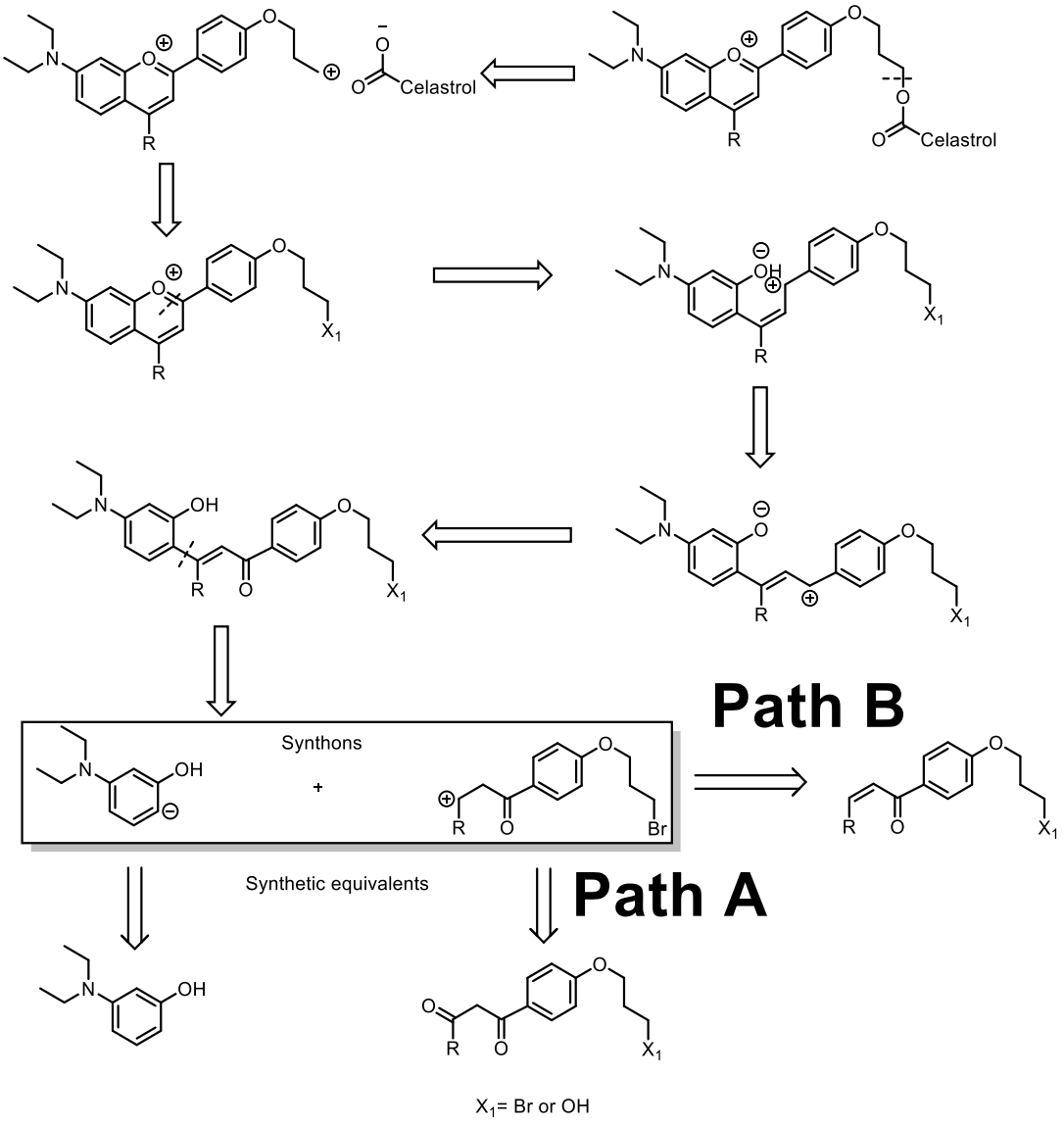


Scheme 69 - Addition of benzotriazole (1.5) to the C4 of the pyrylium ring of the 7-diethylamino-4'-((3 hydroxy)propoxy)flavylium (1.10) with 1.1 equiv. of benzotriazole (1.5) and 1.2 equiv. of NaH in THF at 0ºC to rt for 4 hours. Reaction was performed in a 2mmol scale.

The lack of stability that was demonstrated in the previous statements only exacerbated the necessity of functionalization in the position C4 of the pyrylium ring .In order to bypass the umpolung step an alternative pathway was thought out that consisted on a modification of the reactants in the Robinson Annulation. This conclusion was reached due to the retrosynthetic analysis that, once again proved to very valuable in this work.

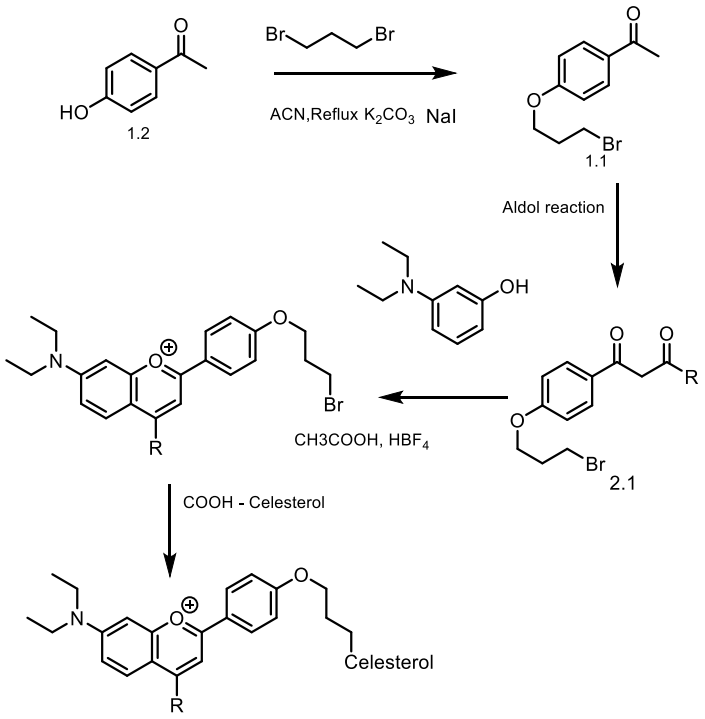
Retrosynthetic consideration on the problematic at hand

The retrosynthetic analysis aids us to understand the transformation that a molecule can undergo during its formation (Scheme 30). Because we have a quite intricate molecular condensation, a couple of secondary's pathways can be found.



Scheme 70 – Second retrosynthetic analysis of the flavylium compound.

The most interesting retrosynthetic disconnection is related to the condensation to generate the flavylium salt. Two separate paths were discovered which are inherently related to one another. The diketone (path A) and chalcone(path B) are reactively similar compound whose synthesis can be, theoretically, achievable. We decided at this time to follow the path A synthesizing the correspondent diketone.



Scheme 71 – General scheme for this synthetic approach using path A - Diketone.

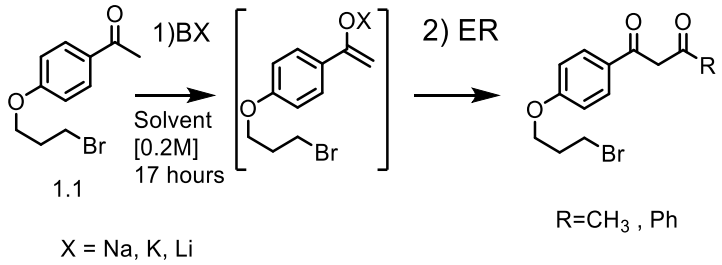
Alternative approach to the C4 functionlization – Diketone synthesis

To obtain the flavylium substituted in the C4 of the pyrylium ring the annulation can occur with a diketone and an aldehyde. In this case the group attached to the pyrylium ring would be the same as one of the sides of the diketone. Another change that would be implied is that a derivative of the salicylaldehyde would no longer be require instead, 3-diethylamino phenol would be used in this transformation.

The first step, like in the first synthetic proposition, consists on the linker coupling to the acetophenone which can be carrying out with ease.

The second step could be achieved by the known aldol reaction to lead for the formation of the diketone. At a first glance there exist some problems with this synthetic path being one of the major the reactivity of the expected product (2.1) which could prove to be troublesome. For

that reason all experiments in this part of the project were carried out with low temperature to increase the control over the reaction conditions[64]. In the first attempt we opted for a base catalyzed aldol reaction with lithium as countercatium to assist in the formation of the enolate (Scheme 32).



Scheme 72 – Aldol reaction of 3 bromo-4-propoxyacetophenone. BX is the necessary base to generate the enolate. ER is the electrophile with the correspondent R group.

Table 20 – All reaction were conducted on a 3mmol scale using 3 bromo-4-propoxyacetophenone (1.1) and 1 equiv. of electrophilic reagent (ER). The reactions were monitored for 17 hours. Reaction temperature 0ºC to rt.

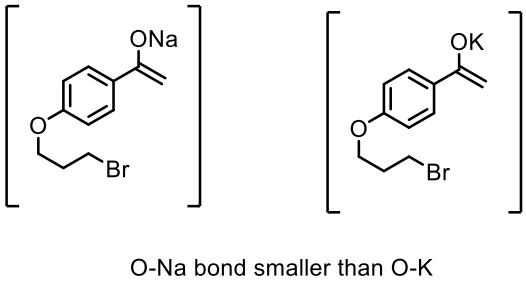
Entry	Base (BX)	Solvent	Electrophile (ER)	Observations
1	$LiCO_3$	DCM	AcOEt	No conversion
2	$(CH_3)_3COK$	DCM	AcOEt	No conversion
3	NaH	THF	AcCOCl	No isolated product
4	NaH	THF	PhCOCl	No isolated product
5	LiHMDA	THF	PhCOCl	No isolated product

This reaction did not show any development during its reaction time. A plausible explanation is that the carbonate is not basic enough to remove the proton of the acetophenone and generate the enolate, and, in addition, the ethyl acetate is not electrophilic enough to be attacked by the enolate (entry 1). The temperature was increased to see if any reaction would occur, having in mind that we had to consider that increasing the temperature could lead to the elimination of the bromine.

In the second experiment potassium *tert*butoxide was used, and still, no reaction occurred since the initial molecules were recovered. This fact demonstrates the importance of the electrophile in this reaction. Because of the non-covalent nature of the O-K bond in relation with the O-Li, the enolate formation could also be compromised[65].

In order to prove the latter affirmation a very hard electrophile was used. Acetyl chloride was freshly distilled right before utilization to prevent the interference with other contami-

nants. Despite all this effort the reaction did not take place. One possible explanation is that the counter cation sodium was not optimal to form the enolate but still better than potassium [66].



Scheme 73 – Enolate formation of a 3 bromo-4-propoxyacetophenone using potassium and sodium bases.

Because we were unsure if the electrophile was being consumed during the reaction we decided, in another attempt, use a hard electrophile that could be followed through TLC. This was of extreme importance because after this experiment we would be able to realize if the problem was in the nucleophilic attack to the electrophile. Thus, benzoyl chloride was used and it remained in solution .The TLC profile remained similar to the previous reactions. The justification to these results is that the enolate did not form and for that reason a stronger base that would lead to a more stable enolate was required.

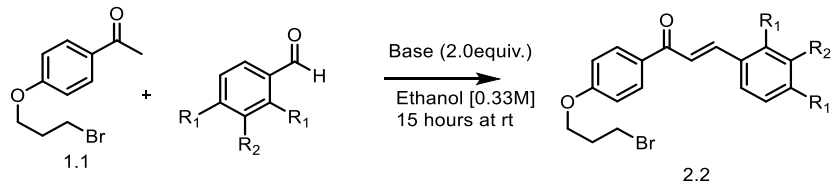
LiHMDA is a super lithium base normally used in aldol reaction due to a factor that is not relevant in this case which is, selective enolation [67]. Some of the reactant was consumed when the benzoyl chloride was added. Even though there was still a lot of acetophenone, and from the TLC plate some other products were formed. Some attempts to isolate these compounds were unsuccessful and only a vestigial amount of diketone was observed.

The latter experiments confirmed the difficulties in accessing the diketone moiety using strong bases. One likely factor that contributed to the outcome of reaction is that the reaction itself would generate the most acidic protons in the alfa position which could then lead, in turn, to side transformations. If the alfa position was substituted perhaps the transformation was possible.

Alternative approach to the C4 functionlization – Chalcone synthesis

We can use retrosynthetic analysis to analyze and find other alternatives for this method. Other possibility that was mentioned in the introduction beforehand is using chalcone instead of the diketone. Immediately there are three notions that came to mind when mentioning this alternative. First the stability of the chalcone should be higher than its correspondent diketone; Second there are a number of examples that report the precipitation of chalcone. And lastly there are a lot more examples of the condensations of the formation of flavyliums from these types of compounds[68].

The initial goal of this new approach was to insert a phenyl ring in the C4 of the flavylium. In order to do that a condensation with 3 bromo-4-propoxyacetophenone and aldehyde was attempted (Scheme 34). It was decided that a base promoted aldol reaction was the best method.



Scheme 74 – Formation of the chalcone using 3 bromo-4-propoxy acetophenone and a benzaldehyde derivative in basic conditions (2.0 equiv.) in ethanol [0.33M] at rt. Purification was by precipitation using methanol.

Table 21 – All reaction were conducted in a 3mmol scale. Absolute ethanol was used as a solvent [0.33M]. Reactions were performed at rt (25ºC).

Entry	Aldehyde	Acetophenone	Base	Yield(%)
1	Benzaldehyde (R_1=R_2=H)	3 bromo-4-propoxy	NaOH	traces
2	Benzaldehyde(R_1=R_2=H)	3 bromo-4-propoxy	$LiCO_3$	traces
3	Benzaldehyde(R_1=R_2=H)	3 bromo-4-propoxy	LiOH	70
4	Benzaldehyde(R_1=R_2=H)	4-propoxy	LiOH	10
5	2,4 dimetoxy benzaldyde	3 bromo-4-propoxy	LiOH	-
6	3 nitro benzaldehyde	3-bromo-4-propoxy	LiOH	-

The first trials involved sodium hydroxide and lithium carbonate that did not lead to the product by precipitation as it was intended (entries 1 and 2). Furthermore the starting material did not seem to have been consumed during the time of the reaction.

When lithium hydroxide was used the reaction with the appropriate acetophenone and benzaldehyde yielded the product (1.12) after precipitation with methanol. In comparison to the previous examples the reaction was quite fast and the yield was very good.

When a method was found for the purification of the chalcone we preceded to try the reaction this time with a hydroxyl functionalized linker (entry 4). The reason for that was that we would like to find out if the condensation to generate the flavylium would also work out with the free alcohol without any need of derivatization.

The same exact reaction was carried out with the exception of the equivalents of LiOH which were augmented due to the possibility of abstraction on the free alcohol. This time the reaction was quite slower and the precipitation did not occur normally with methanol. Several other solvents were experimented and it was found that after, an aqueous work-up, to neutralize the medium the product precipitated with diethyl ether. One possible explanation was that the product did not precipitate if it is on an anion form – which was the case pre work up. The yield was also significantly lower than the bromine case.

Now that we had a method to introduce the phenyl group we tried other aldehydes which could introduce other electronic proprieties into the system – nitro and metoxy substitution (entry 5 and 6).

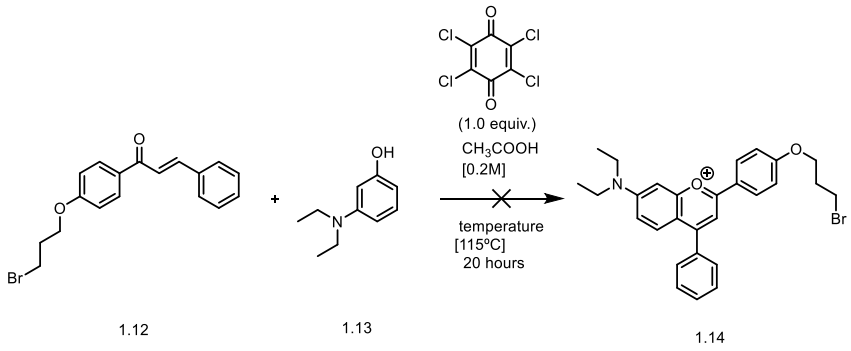
Two different aldehydes were tested using this methodology however precipitation was not successful in either case. A fact that was recurring in both compounds were they not com-

pletely pure and for that reason the purification proved to became difficult. Another important fact to mention is that no full conversion of the initial regents was achieved and in the purification by flash chromatography the compounds seem to degradated.

At this time we questioned whether the condensation reaction would yield the desired product and for that reason we decided to pursue the enhancement of the aldehyde scope later.

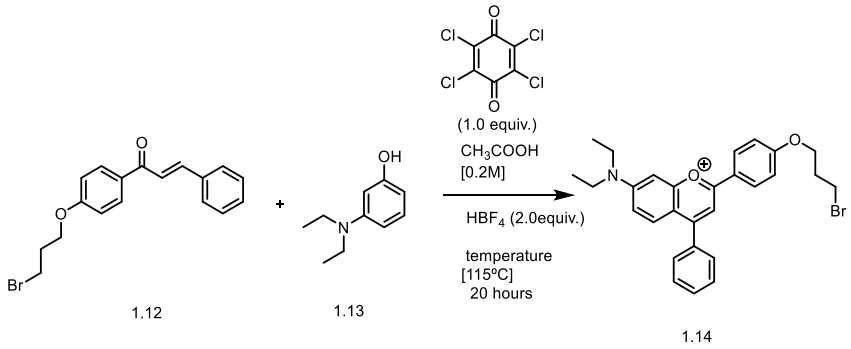
Chalcone / phenol condensation in mild oxidant conditions

The condition for the first condensation using the chalcone and the phenol were acetic acid at reflux without the addition of a strong acid. This experiment was adapted from literature that used o-chloranil to promote the generation of the flavylium salt[38](Scheme).



Scheme 75 – Acidic condensation of a chalcone (1.12) with 3-diethylaminophenol (1.13) with o-chloranil (1.0equiv.) in acetic acid [0.2M] at 115ºC. Fluorescent product was not found in the TLC plate. Reaction was performed in a 2mmol scale.

After the analysis of this result we did not found any product however we arrived to very important information. Unlike previous reaction that the product precipitated using diethylether and ethylacetate, this time the impurities and starting material did. A glimpse on the TLC plate we could see that no fluorescent product was generated during the procedure.



Scheme 76 - Acidic condensation of a chalcone (1.12) with 3-diethylaminophenol (1.13) and o-chloranil (1.0equiv.), in acetic acid [0.2M] and HBF_4(2.0equiv.) at 115ºC. Reaction was performed in a 2 mmol scale.

There was a high possibility that the reaction required a strong acid to be successful[69] and for that reason we used HBF4- to be able to compare with previous reactions of the flavylium cation formation. There was a significant change in the color of to a much brighter red tone. Furthermore, despite the acknowledging that a fluorescent product was generated during the reaction we still could not successfully isolate the flavylium in this case. A variety of organic solvents were used to precipitate the components of the reaction however only small amount of starting material were isolated and identified.

A breakthrough was reached when, during the washing of a flask, occurred precipitation of a red oil with a yellow supernatant. After the analysis of the oil we could identify the expected flavylium salt with a bromine carbon linker. This result was corroborated with high resolution mass spectra. By serendipity we discovered that the product precipitated using water leaving the other impurities in the supernatant.

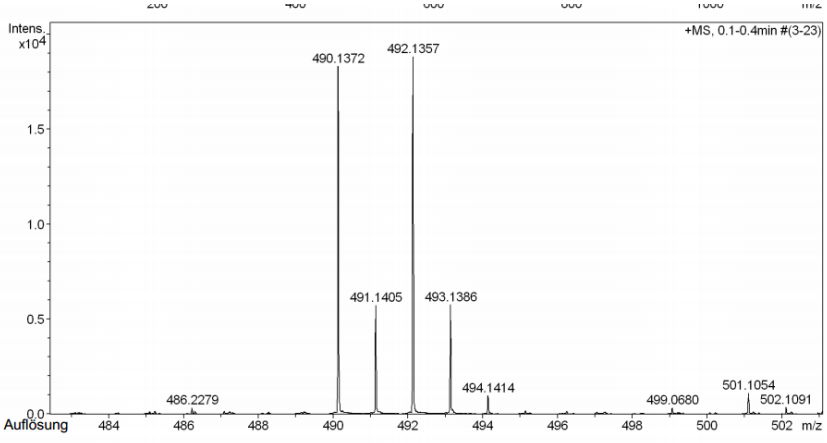


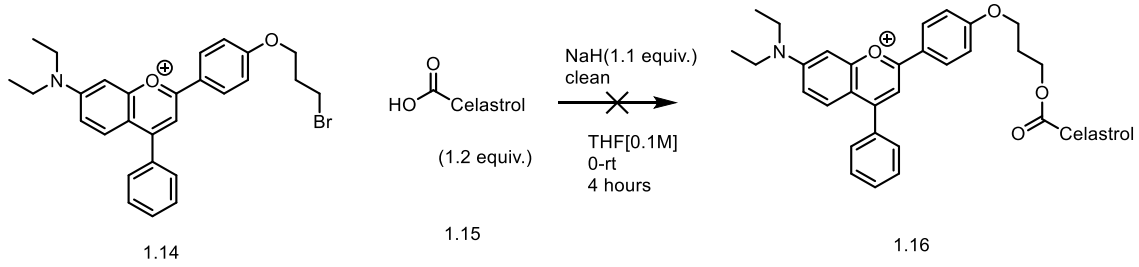
Figure 78 – Mass spectra of the flavylium salt (1.14).

This was the first flavylium salt we successfully synthesized and that brought other possibilities to the table. One of them consists of the possibility to generate the flavylium in larger quantities.

The initial idea now that the correct flavylium had been synthesized was to connect it to the celastrol using its carboxylate salt(Scheme 37). A necessary disclaimer about all reaction conducted with celastrol is that due the high price of compound (700 euros *per* 50mg) the scale of the reaction was quite small (around 0.1mmol).

Celastrol coupling attempt using a carboxylate salt

There are numerous ways to generate the carboxylate salt, yet, in this case, there were two situations that we needed to control. The first was that high basic medium could react with celastrol changing its characteristics which are utterly important for this work. The other reason which is also connected the pH of the medium is that the flavylium could also open if the medium is too alkaline. The thought process to move pass from this hurdle was by using just 1 equiv. of a strong base. The first experiment was done with sodium hydride without it being on a mineral solution. The reasoning behind it is that besides the reaction being much cleaner without the oil it also allows for the exact measure of the amount which is in the flask after it being washed.



1.14 1.15 1.16

Scheme 77 – Reaction of celastrol (1.15) carboxylate with flavylium salt with a halogenated linker (1.14). NaH (1.1 equiv.) were used with THF [0.1M] at rt. Reaction was performed in a 0.1 mmol scale.

This approach did not yield the expected product (1.16) or any other for that matter. The pH of the solution remained acidic which could sign that the flavylium still had some solvent in it. Other possible assertion that could be made is that perhaps there was not enough sodium hydride to completely remove the proton from celastrol due to low scale of the reaction. The process of washing the sodium hydride is really meticulous meaning that the amount of base could have been miss weight in, especially at lower scale reaction.

In this reaction it was decided to use celastrol in excess in order to not have competing precipitation amongst both flavylium salts – the product and the starting material. Experimentally there was no difference in the TLC pattern from the carboxylate salt from celastrol and its neutral compound. This is clearly an argument about the inexistence of activation of the base to the celastrol compound.

III-Future Prospects

As it was evident throughout the course of this work is that the final objective has not been yet achieved. What is important at this point is there are a several number of alternatives / solutions that could be employed in order to reach the final target which is so close experimentally speaking.

In this final step of our synthetic approach, because a substitution of a bromine is taking place, a method that involved its exchange with a better group could be tried out. This is a very interesting point to make because, that solution was already developed in the first step of the synthesis. A catalyst like sodium or potassium iodide could catalyze the reaction and prove to be successful.

A variation of the base could also be beneficial to the generation of the carboxylate anion. A couple of examples are potassium carbonate or cesium carbonate which main difference is their solubility in most organic solvents. As a matter of fact one could exploit the low solubility of potassium carbonate in this case because of the necessity of not having a very alkyline medium. By the otherhand one could also make a case for the of cesium carboxylates being extremely effective in SN2 type substitution of halides, which is exactly what is intended in this transformation [70].

In regards to one of the most essential parts of the work, the flavylium formation, the condensation could also be experimented with other strong acids such as percholoric acid. It was also unexpected that the condensation did not occur when the alcohol linker was employed. One possible resolution to this would be protecting the alcohol group.

In regards to the flavylium salts which are being targeted in this work is also vital that fotochemical tests are carried out when they are synthesized. These tests could led to interesting conclusions about the proprieties of the compounds, which, in term, could lead to applications in other topics.

Since the synthesis with the bromine worked, one could also access the alcohol moiety by substitution of the halogen. After that step, the esterification by activation with a coupling reagent could be experimented.

On the topic of the synthesis of chalcones a variety of aldehydes could still be studied. Even the simple acetaldehyde would yield the 4 methyl substituted flavylium and, after that, we could infer the role of conjugation in stabilization of the flavylium in the position 4. Other pro-

cedures of the chalcone formation could also be pursuit to solve the synthesis of using substituted benzaldehydes.

In the first steps of introduction of a carbon linker onto a hydroxyl acetophenone one possible way that the method could be extensive improved is by lowering the reaction temperature (acetronitrile reflux). Other solvents systems could be considered because the high temperature with halides leads to elimination which was the major problem in those syntheses.

Perhaps the most important part of this quest is the final transformation of the flavylium to the selected drug. This should be of upmost priority since the final goal of molecular recognition using fluorescent probes demands it.

IV-Conclusions

During the course of this work a great deal of knowledge was acquired.

One of the most important skills that were acquired in this work is not easily apparent due to the fact it did not yielded concrete results. We are talking about the numerous times the precipitation process was attempted and failed. Because most of the examples of similar compounds yielded solids by precipitates we were fairly sure that we could purify the compounds by the same technique. The number of eluents and different proportion of solvents that were tried out far exceed the hundreds with little to no avail in the purification of some flavylium salts.

From all the failed results an invaluable resource was acquired that is utterly important when doing investigation – discover alternatives. That was probably the most precious skill that came out of this work. Sometimes is easy to characterize a bad outcome as non- interesting and that is certainly a mistake. If by any reason all the reaction which turn out bad would be discarded, the technological advancement which we are so proud nowadays would be much prevalent.

In the early parts of this work we were able to optimize a method for the insertion of a 3 carbon halogenated linker by phenol moieties. The reaction was chemoselective and no alkylation of alfa position of the ketone was detected. It was also showed that the reaction occurred with stoichiometric amounts of catalyst.

Several different ways to reach the flavylium moiety were also tested out with most of them being able to deliver the fluorescent product. Through the process of experimentation crucial factors such as the presence of a strong acid and high temperature were identified as helpful for the transformation. Another reagent which proved to increase the reaction rate in the condensation reaction was acetic anhydride, whose slow addition lead to a change of color in the solution.

Still on the topic of the flavylium synthesis an alternative to the normal purification procedure had to be develop – precipitation followed by filtration. The fact the flavylium had a very low particle size it meant that it passed completely by the pores of the filtration systems. The alternative explored was centrifugation that, as far we are aware, had not been done with flavylium salts up to this point.

Other progresses were also made in alternative ways in the generation of the flavylium salts. The synthesis of diketones and chalcones had to be attempted and only the latter afforded

the expected product. Nevertheless the diketone synthesis proved to be challenging as it lead to unstable product and proved some concepts about the aldol condensation. In terms of chalcones most of them were proved to be unstable in silica.

In the condensation of chalcones and 3-diethylamine phenol to afford the 4 substituted pyrylium ring was done in the presence of o- chloranil, usually used as a oxidant, proved to enhance the reaction speed. This fact was seen in one of the first articles on the synthesis of flavylium it has not been the favored method in their formation.

The coupling between the molecular fluorescent probe, flavylium and the pharmaceutical compound Celastrol was not successful however, a very important piece of information was proven that could be of immense value in future works – the activation of the carboxylic acid in mild conditions and temperatures is not easily achievable, even with strong bases, and for that reason the temperature could play a major role in the transformation.

A very important detail that should be underlined is the motivational part that is inherent t this work. Because the final destination is very interesting in terms of the possible effects of the conclusion of this work, it means that, even if all the experiments do not reach the expected product they still are important pieces in the general outline of the project.

Another important feature of this theme is that its applicability is very immediate because after the target compounds are synthesized they can be used in tests to prove its effects.

Celastrol and its derivatives have a huge amount of therapeutical potential which exacerbates the potential of this work itself. If the coupling between the molecular fluorescent probe and celastrol does occur, there is a possibility it can also ensue with celastrol derivatives.

The major prospective that should be highlight is that the final objective consists on the understanding of intracellular processes which are directly connected to the most urgent problems which are being investigated nowadays – disease treatment and understanding.

V-Experimental part

Disclaimer

All reagents were used as received from commercial suppliers unless otherwise stated. Reaction progress was monitored by thin layer chromatography (TLC) performed on aluminium plates coated with silica gel F_{254} with 0.2 mm thickness. Chromatograms were visualized by fluorescence quenching with UV light at 254 nm or by staining using potassium permanganate/ Ninhydrin. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Carlo Erba). Neat infra-red spectra were recorded using a Perkin-Elmer Spectrum two FT-IR spectrometer. Wavenumbers (**vmax**) are reported in cm^{-1}.

All ^{1}H NMR and ^{13}C NMR spectra were recorded using a Brucker ARX400 .Chemical shifts were given in parts per million (ppm, δ), referenced to the solvent peak of $CDCl_3$, defined at δ = 7.26 ppm (^{1}H NMR) and δ = 77.16 (^{13}C NMR). Coupling constants are quoted in Hz (*J*). 1 and 13C splitting patterns were designated as singlet (s), doublet (d), triplet (t), quartet (q), sextet(sext), septet (sept). Splitting patterns that could not be interpreted or easily visualized were designated as multiplet (m) or broad (br).

Experimental data of the synthesized compounds

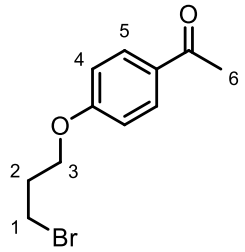


Figure 79 - 1-(4-(3-bromopropoxy)phenyl)ethan-1-one.

The 1-(4-(3-bromopropoxy)phenyl)ethan-1-one was isolated after silica gel flash chromatography purification in 84% yield as a yellow oil.

Results in accordance with literature [71]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93 (d, *J*=8.4 Hz, 2H, *H*5), 6.94 (d, *J*=8.8Hz, 2H, *H*4), 4.17 (t, *J*=5.6Hz, 2H, *H*3), 3.60 (t, *J*=6.4Hz, 2H, *H*1), 2.55 (s, 3H, *H6*), 2.34 (q, *J*= 6.4Hz. 2H, *H2*)

^{13}C NMR (151 MHz, $CDCl_3$) δ = 196.89, 162.71, 130.76, 130.70, 130.67, 114.53, 114.30, 77.48, 77.16, 76.84, 65.63, 32.25, 29.81, 26.49, 2.19.

IR (neat, cm^{-1}): 2936, 2882, 1672, 1598, 1508.

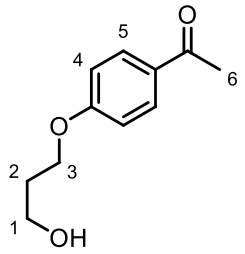


Figure 80 - 1-(4-(3-hydroxypropoxy)phenyl)ethan-1-one.

The 1-(4-(3-hydroxypropoxy)phenyl)ethan-1-one was isolated after silica gel flash chromatography purification in 82% yield as a yellow oil.

Results in accordance with literature[72]

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.91 (d, *J*=8.8 Hz, 2H, *H*5), 6.93 (d, *J*=8.8Hz, 2H, *H*4), 4.18 (t, *J*=6.0Hz, 2H, *H*3), 3.86 (t, *J*=6.0Hz, 2H, *H*1), 2.54 (s, 3H, *H6*), 2.06 (q, *J*= 6.0 Hz. 2H, *H2*)

^{13}C NMR (151 MHz, $CDCl_3$) δ = 197.03, 162.92, 130.74, 114.27, 65.66, 59.97, 32.01, 26.46.

IR (neat, cm^{-1}): 3408, 2949, 2882, 1671, 1666, 1509.

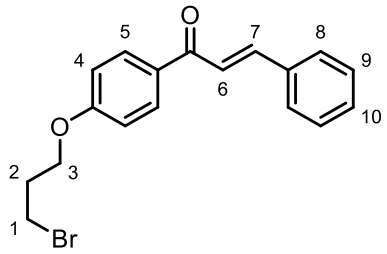


Figure 81 - (E)-1-(4-(3-bromopropoxy)phenyl)-3-phenylprop-2-en-1-one.

The 1-(4-(3-bromopropoxy)phenyl)ethan-1-one was isolated after precipitation in 60% yield as an oil. Results in accordance with literature[73].

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.04 (d, *J*=8.8 Hz, 2H, *H*5), 7.79 (d, *J*=15.6Hz, 1H, *H7*), 7.65 (d, *J*=3.8Hz, 2H, *H*8), 7.55 (d, *J*=15.6Hz, 1H, *H*6), 7.42 (m, 3H, *H9-H10*), 7.00 (d, *J*= 8.8 Hz. 2H, *H4*), 4.21 (t, *J*=5.8Hz, 2H, *H*3), 3.63 (d, *J*=6.4Hz, 2H, *H1*), 2.37 (q, *J*= 6.0 Hz. 2H, *H2*).

IR (neat, cm^{-1}): 2929, 2882, 1672, 1592, 1508.

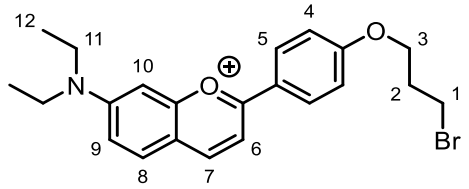


Figure 82 - 2-(4-(3-bromopropoxy)phenyl)-7-(diethylamino)chromenylium.

The 2-(4-(3-bromopropoxy)phenyl)-7-(diethylamino)chromenylium was characterized in an oil with residual solvent.

Structure assignment was done using 2D NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.79 (d, *J*=8.0 Hz, 1H, *H*7), 8.38 (d, *J*=8.8 Hz, 2H, *H*5), 8.05 (d, *J*=8.0Hz, 1H, *H*8), 8.00 (d, *J*=9.6Hz, 1H, *H*9), 7.49 (d, *J*=9.2Hz, 1H, *H6*), 7.39 (m, 1H, *H10*), 7.28 (d, *J*=8.8Hz, 2H, *H*4), 4.28 (d, *J*=5.6Hz, 2H, *H3*), 3.71 (m, 6H, *H1/H12*), 2.31 (q, *J*= 6.0 Hz, 2H, *H2*), 1.25 (t, *J*= 6.4 Hz, 6H, *H11*).

^{13}C NMR (151 MHz, $CDCl_3$) δ = 166.08, 163.55, 158.98, 149.06, 130.49, 130.43, 130.01, 122.01, 115.77, 114.27, 95.92, 65.67, 31.64, 31.06, 30.98, 12.42, 0.09.

IR (neat, cm^{-1}): 3029, 2959, 2916, 1738, 1365.

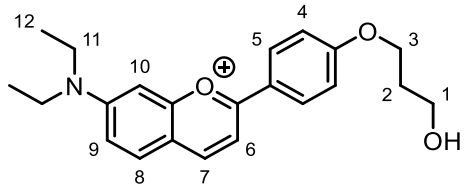


Figure 83 - 7-(diethylamino)-2-(4-(3-hydroxypropoxy)phenyl)chromenylium.

The 7-(diethylamino)-2-(4-(3-hydroxypropoxy)phenyl)chromenylium was characterized in an oil with residual solvent.

Structure assignment was done using 2D NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.74 (d, J=8.0 Hz, 1H, *H*7), 8.34 (d, J=8.8 Hz, 2H, *H*5), 8.02 (d, J=8.0Hz, 1H, *H*8), 7.97 (d, J=9.6Hz, 1H, *H*9), 7.45 (d, J=9.2Hz, 1H, *H6*), 7.34 (m, 1H, *H10*), 7.22 (d, J=8.8Hz, 2H, *H*4, 3.70 (m, 6H, *H1/H12*), 1.91 (q, J= 6.0 Hz, 2H, *H2*), 1.24 (t, J= 6.4 Hz, 6H, *H11*).

^{13}C NMR (151 MHz, $CDCl_3$) δ =166.33, 164.13, 159.08, 156.11, 149.14, 132.46, 130.58, 118.37, 118.17, 115.86, 108.51, 96.03, 65.67, 57.21, 45.68, 31.98.

IR (neat, cm^{-1}): 3393, 3029, 1738, 1365.

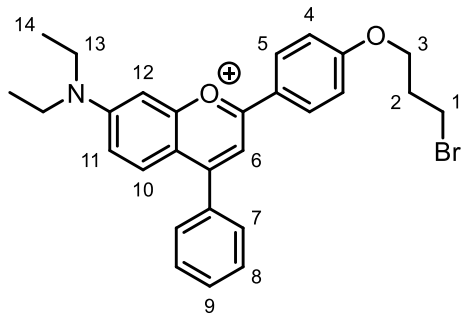


Figure 84 - 2-(4-(3-bromopropoxy)phenyl)-7-(diethylamino)-4-phenylchromenylium.

Structure assignment was done using 2D NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ =8.76(m), 8.45(m), 7.67(m), 7.22(m), 4.23(m), 3.70(m), 3.64(m), 2.30(m), 1.22(m).

HMRS: calculated for: : $C_{28}H_{29}BrNO_2$+ :490.1376 found: 490.1372

IR (neat, cm^{-1}): 3188, 2969, 2603, 1739, 1600, 1483.

Experimental procedures

V.1-Synthesis 3, bromo-4-propoxycetophenone

A mixture of 4-hydroxyacetophenone (5 mmol scale) with previously crushed potassium carbonate (5 equiv.) and sodium iodide (x equiv.) were dissolved in undistilled ACN PA [0.1M] and stirred for 30 minutes .After that time 1,3 dibromopropane (3 equiv.) was added and the solution was allowed to warm until reflux (80ºC). After 18 hours the mixture was cooled to room temperature and diluted with ethyl acetate (15 mL) and filtered using a Hirsh funnel. Afterwards the solution was extracted with brine (2 x 10 mL). The of organic phase was dried over anhydrous sodium sulphate, filtered and the solvent removed on a rotary evaporator. A yellow oil was obtained and purified by column chromatography on silica gel (7-1 Hexane, Ethyl Acetate).

V.2-Synthesis of the flavylium salt by Robinson Annulation of 3-bromo 4-propoxycetophenone and 4-diethylaminosalicylaldehyde

A mixture of 3-bromo-4-proproxyacetophenone (2mmol scale) and 4-diethyl amino salicylaldehyde (1.0 equiv.) were added to 1.25 mL of Acid Additive and glaciar acetic acid [0.2M] at room temperature .The solution was heated to 100 ºC and stirred for 18 h. After that period diethyl ether was added and a dark pink precipitate was centrifuged.

V.3-Synthesis of 3-hydroxy 4-propoxycetophenone

A mixture of 4-hydroxyacetophenone (5mmol scale) with previously crushed potassium carbonate (5 equiv.)and sodium iodide (x equiv.) were dissolved in wet ACN [0.1M] and stirred for 30 minutes .After that time 3-chloro-1-propanol(3 equiv.) was added and the solution was allowed to warm until reflux (80 ºC). After 18 hours the mixture was cooled to room temperature and diluted with ethyl acetate (15 mL) and filtered using a Hirsh funnel. Afterwards the solution was extracted with brine (2 x 10 mL). The of organic phase was dried over anhydrous sodium sulphate, filtered and the solvent removed on a rotary evaporator. A yellow oil was obtained and purified by column chromatography on silica gel (4-1 Hexane Ethyl Acetate).

V.4-Synthesis of the flavylium salt by Robinson Annulation of 3-bromo-4-proproxyacetophenone and 4-diethyl amino salicylaldehyde

A mixture of 3-bromo-4-proproxyacetophenone (2mmol scale) and 4-diethyl amino Salicylaldehyde (1.0 equiv.) were added to 1.25 mL of Acid Additive and glaciar acetic acid[0.2M] at room temperature .The solution was heated to 100 ºC and stirred for 18 hours. After that period diethyl ether was added and a dark pink precipitate was centrifuged.

V.5-General procedure for the addition of benzotriazole addition to flavylium salt.

Experimental procedure was addapted from literature with no modification[40].

V.6-General procedure for aldol reaction in basic conditions

In a previously dried flask under nitrogen atmosphere 3 bromo-4-propoxycetophenone was dissolved in the appropriate solvent. Afterwards of Base (2.0 equiv.) was added and the solution was stirred and put at in an ice bath for 30 minutes. At this point the electrophile (1.0 equiv.) was added and allowed to warm up to the described temperature. After 18 hours the reaction was quenched with methanol and extracted (2x) with brine. The organic phase was dried with anhydrous sodium sulfate and filtrated using a hirsh funnel. The solvent was evaporated and passed through a flash silica column.

V.7-General procedure for the synthesis of chalcones using 3 bromo-4-propoxycetophenone

In a previously dried flask the appropriate base (2.0 equiv.) was added to 3 bromo-4-propoxycetophenone and dissolved in ethanol, [0.25M]. The corresponding benzaldehyde (1.0 equiv.) was then added and the mixture was stirred to 10 hours. After evaporation of the solvent in reduced pressure, methanol was added to precipitate the product. The mixture was washed with hexane to remove impurities.

V.8-General procedure for the 4 substituted flavylium salts using Robinson annulation.

A mixture of 3 bromo-4-proproxyacetophenone (2 mmol scale), 3-diethyl amino phenol (1.0 equiv.) and o-chloranil (1.0 equiv.) were added to 1.25mL of Acid Additive and glaciar acetic acid [0.2M] at room temperature .The solution was heated to 100^{o}C and stirred for 18 hours. After that period distilled water was added and pink needles were centrifuged. The solution was then recrystallized using diethyl ether to afford the clean product.

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Appendix

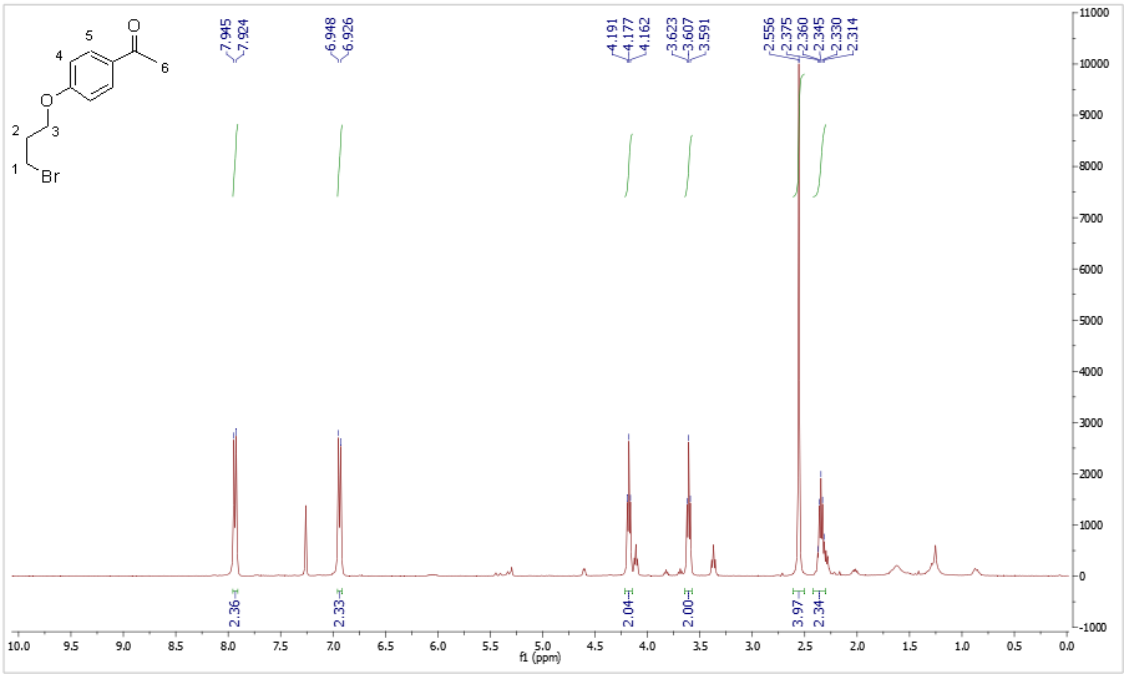

Figure 85 – ^{1}HNMR spectra of (E)-1-(4-(3-bromopropoxy)phenyl)-3-phenylprop-2-en-1-one.

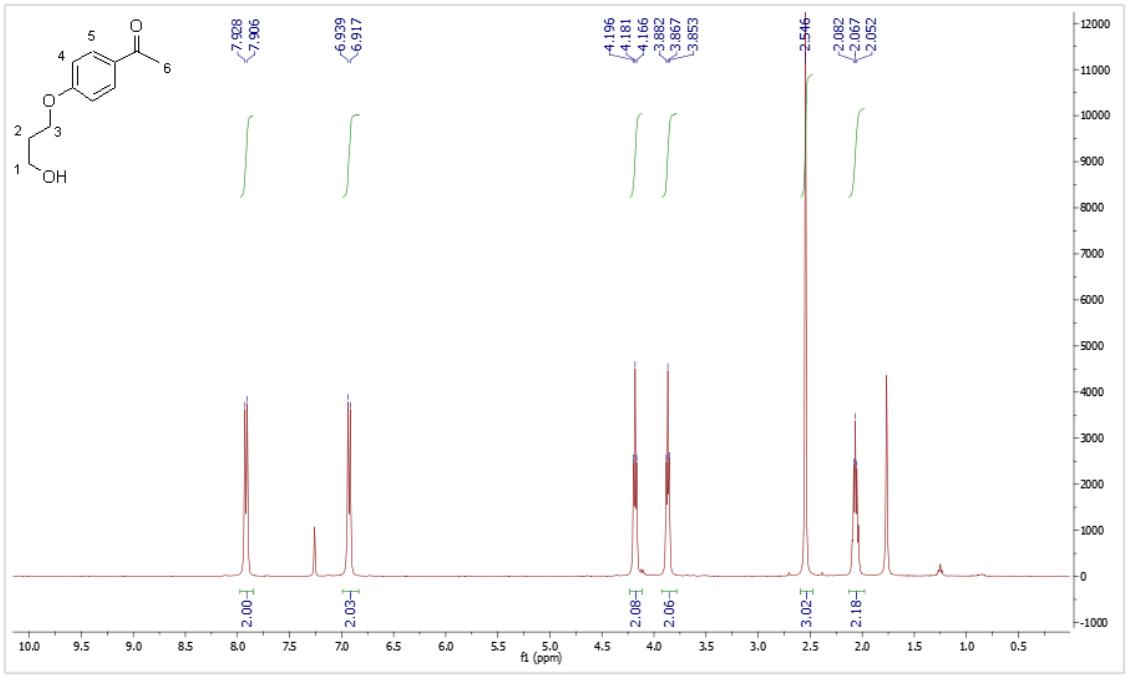

Figure 86 – ^{1}HNMR spectra of 1-(4-(3-hydroxypropoxy)phenyl)ethan-1-one.

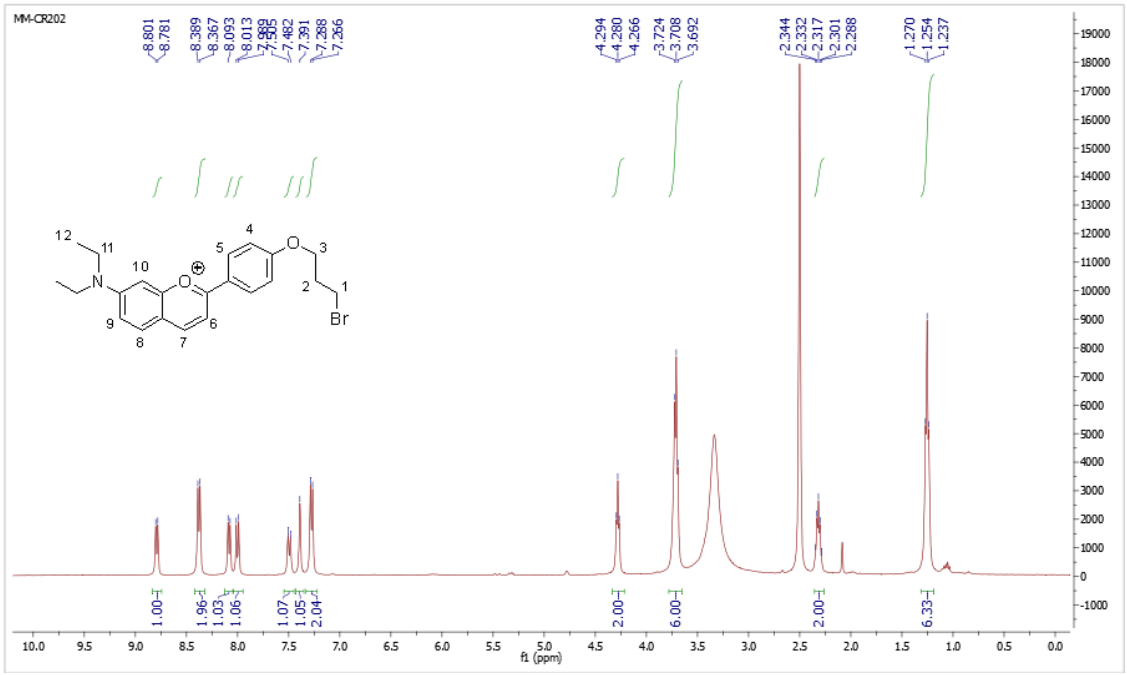

Figure 87 – ^{1}HNMR spectra of 2-(4-(3-bromopropoxy)phenyl)-7-(diethylamino)chromenylium.

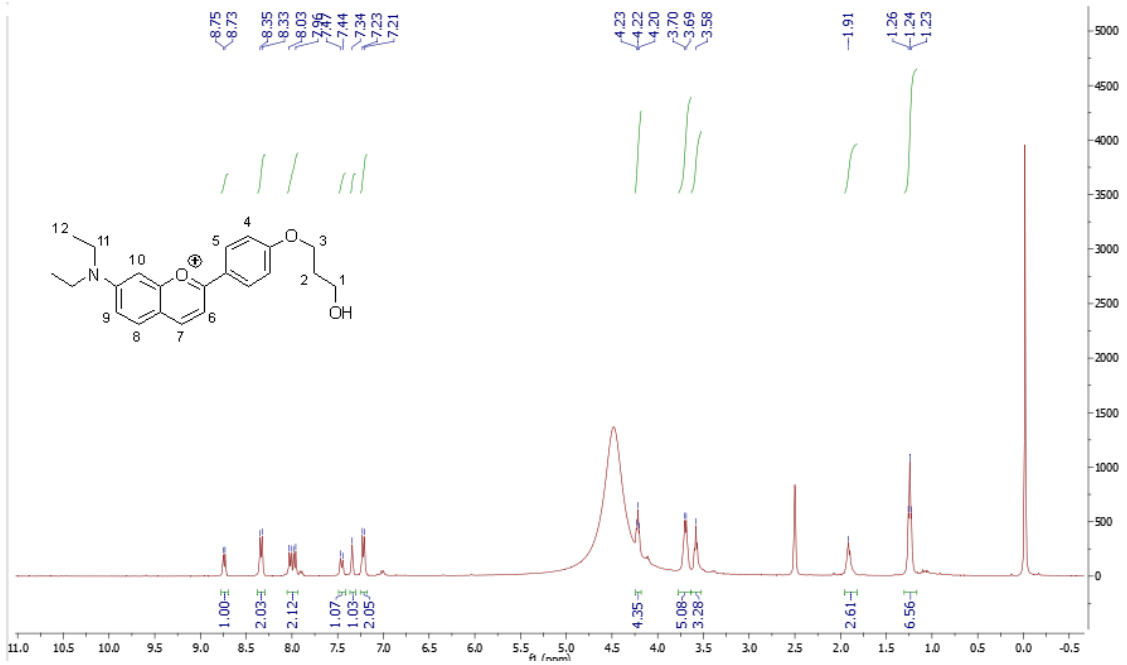

Figure 88 – ^{1}HNMR spectra of 7-(diethylamino)-2-(4-(3-hydroxypropoxy)phenyl)chromenylium.

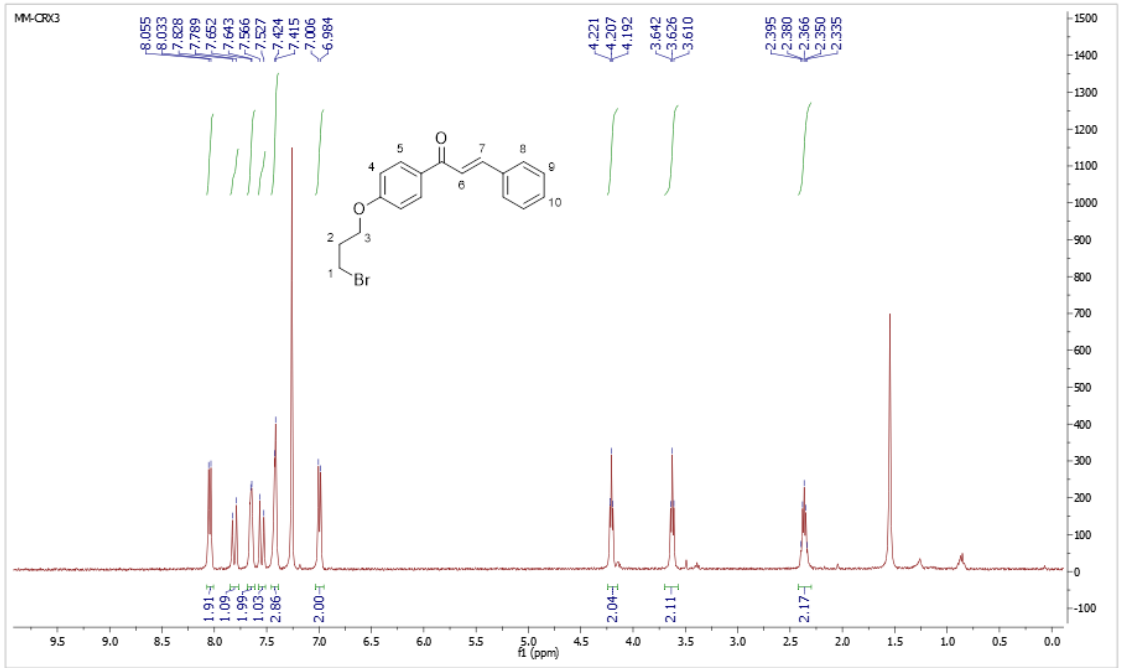

Figure 89 – [1]HNMR spectra of (E)-1-(4-(3-bromopropoxy)phenyl)-3-phenylprop-2-en-1-one.

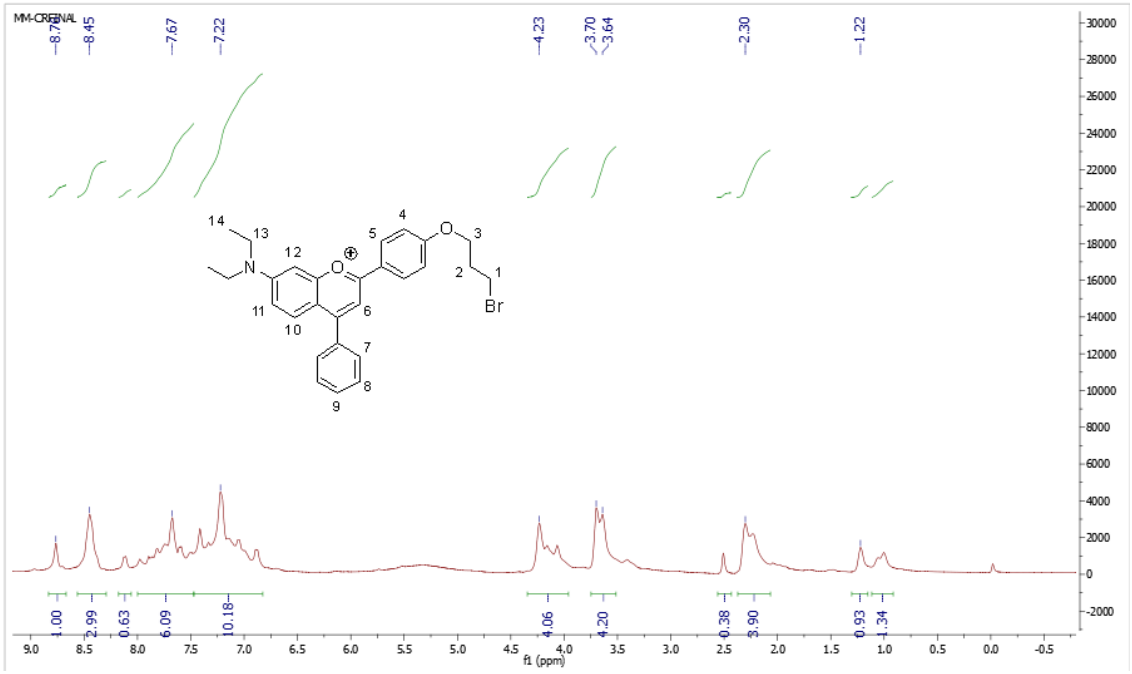

Figure 90 – [1]HNMR spectra of - 2-(4-(3-bromopropoxy)phenyl)-7-(diethylamino)-4-phenylchromenylium